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Parallel Sessions

Sunday May 22, 2022

Parallel Session 1 – Multiple dimensions of dopamine signaling: New technologies and novel insights

Diversity of dopamine circuits in reward and aversion

Stephan Lammel[1]
[1]University of California Berkeley

The mesocorticolimbic dopamine (DA) system, composed of DA neurons in the ventral tegmental area (VTA) projecting to nucleus accumbens and prefrontal cortex, has been intensively studied because of its importance in reward processing and motivated behavior. Importantly, while VTA DA neurons were thought to represent a homogeneous cell population, recent research has demonstrated a much greater diversity of DA cell type and function than had been previously supposed. Accordingly, VTA DA neurons encode much more than reward and also contribute to aversive behaviors. How DA neurons could mediate both reward and aversion is an important goal of the research in my lab. In my presentation, I will discuss recent work that has elucidated the circuit architecture and function of distinct mesoaccumbal DA subcircuits (Yang et al., 2018; Neuron; de Jong et al., 2019). Collectively, our results suggest that we need to develop a new perspective on the DA circuitry that will guide future treatment strategies for addiction and other neuropsychiatric disorders where dysfunction of the neural systems underlying motivated behaviors have been strongly implicated.

Imaging striatal dopamine release with a near-infrared fluorescent nanosensor

Markita Landry[1]
[1]University of California, Berkeley

Neuromodulation plays a critical role in brain function in health and disease. New optical tools are needed that can image neuromodulation with high spatial and temporal resolution, which will add an important new dimension of information to neuroscience research. I will demonstrate the use of a catecholamine nanosensor with fluorescent emission in the 1000-1300 nm near-infrared window to measure dopamine (DA) transmission in acute brain slices. These near-infrared catecholamine nanosensors (nIRCats) can detect DA efflux in the brain extracellular space driven by electrical or optogenetic stimulation. Spatial analysis of electrically-evoked signals reveal dynamic regions of interest 2 microns in size in which transients scale with simulation intensity. I will also show that the chemically synthetic molecular recognition elements of nIRCats permit measurement of DA dynamics in the presence of DA D2 receptor agonists and antagonists, revealing heterogeneity in D2 autoreceptor modulation of presynaptic DA release. These data suggest nIRCats and other nanosensors of this class can serve as versatile new optical tools to report dynamics of extracellular neuromodulation in brain tissue.

Imaging intracellular responses to dopamine in vivo at single cell resolution

Tianyi Mao[1]
[1]Vollum Institute

Great strides have been made towards detecting dopamine (DA) releases. However, in order to understand how DA exerts its function, it is also necessary to visualize how individual cells respond to dopamine in live tissue and during behavior. We have focused on imaging the cAMP/PKA pathway, which is a key downstream mediator of dopamine signaling. In this meeting, we will present our recent development of novel PKA and cAMP fluorescence sensors combined with modern microscopy to image intracellular responses to endogenous dopamine release at single-cell and subcellular resolutions in brain slices and behaving animals. We will also present the distinct cellular and subcellular responses triggered by dopamine versus norepinephrine. Furthermore, the interaction of dopamine and opioid systems has been extensively demonstrated at the circuit, systems, and behavioral levels. However, little is known about whether and to what extent opioids may interact with dopamine at the level of intracellular signaling. We will present evidence that dopamine-induced PKA signaling is modulated by distinct opioid agonists in a manner that is specific to circuit contexts.

Investigating the role of VTA-dopaminergic neurons in the social motivation

Benoit Girard
[1]University of Geneva

Although some evidence has suggested the rewarding nature of social interaction and neuroimaging studies and fiber photometry analyses have demonstrated that social stimuli recruit neurons from the mesocorticolimbic, how dopaminergic neurons in the ventral tegmental area (VTA-DA) signal social context and interactions remains largely unknown. We used in vivo electrophysiological recordings in freely moving mice to investigate which aspects of interaction with a conspecific are rewarding and whether the activity of dopaminergic neurons guides social reinforcement learning via social prediction error. Interestingly, VTA-DA neurons are activated during conspecific interactions and play a crucial role as reinforcement learning signal by encoding social prediction error, as observed in a social instrumental task. We are here improving knowledge to better understand the heterogeneous responses of DA neurons during social interactions and improving behavioral approaches to refine unbiased quantitative and qualitative analysis of social interactions. Our data are not only important for understanding how social interaction is processed by the brain, but they also open the door to possible mechanisms underlying social deficits in psychiatric disorders. Indeed, although a growing number of papers hypothesize that deficits in reward prediction error in a social context can explain social deficits in psychiatric disorders, whether VTA-DA neurons support social prediction in still largely unknown.

Parallel Session 2 – Neuromelanin-sensitive MRI: A method to investigate the integrity and function of catecholamine systems in the human brain

Neuromelanin-Sensitive MRI: A Novel, Non-Invasive Proxy Measure of Dopamine Function in Psychiatric Illness

Clifford Cassidy[1], Fabio Zucca[2], Ragy Girgis[3], Jodi Weinstein[4], David Sulzer[3], Luigi Zecca[2], Anissa Abi-

Dargham[4], Guillermo Horga[3]
[1]University of Ottawa, [2]National Research Council of Italy, [3]Columbia University, [4]Stony Brook University

Background and Aim: Neuromelanin-sensitive magnetic resonance imaging (NM-MRI) has proven to be a sensitive neuroimaging marker for degeneration of dopamine neurons in Parkinson's disease but its utility as a marker of dopamine function in non-neurodegenerative conditions remains unclear. Methods: We validated NM-MRI in the substantia nigra (SN) against concentration of NM in post-mortem human midbrain tissue samples (n=7). We correlated NM-MRI in the SN against a Positron Emission Tomography (PET)-based measure of dopamine release capacity (based on amphetamine-induced displacement of the radiotracer [11C]raclopride) obtained in individuals without a neurodegenerative condition (n=18). To test its utility as a proxy for psychosis-related dopamine dysfunction, we collected data in 33 unmedicated individuals with schizophrenia, 25 individuals at high risk for psychosis, and 50 healthy controls. For voxelwise analyses, we used a permutation-based method for correction for multiple comparisons. Results: NM-MRI signal intensity in postmortem midbrain specimens correlated with regional NM concentration even in the absence of neurodegeneration. Voxelwise analysis within the SN in vivo revealed a cluster where NM-MRI signal-to-noise positively correlated with striatal dopamine release capacity (rho=0.55, $p<0.05$). Voxelwise analyses in the psychiatric populations identified overlapping clusters where higher NM-MRI signal-to-noise in the SN correlated with more severe psychotic symptoms both in patients with schizophrenia and in individuals at clinical high risk (conjunction $p<0.0001$). Conclusions: Our results indicate that NM-MRI signal reflects dopamine system function and captures a psychosis-related phenotype. Future work should evaluate the utility of NM-MRI as a predictive biomarker for treatment response or illness conversion in at-risk populations.

Structure, Synthesis and Role of Neuromelanins in Brain Aging and Parkinson's Disease

Luigi Zecca[1], Ioannis Isaias[2], Luigi Casella[3], Guillermo Horga[4], Clifford Cassidy[5], Gianni Pezzoli[6], David Sulzer[4], Fabio Zucca[1]
[1]National Research Council of Italy, [2]Julius Maximilian University, [3]University of Pavia, [4]Columbia University, [5]University of Ottawa, [6]Parkinson Institute

We have demonstrated that neuromelanins (NM) are a family of compounds occurring in many regions of human brain. They especially accumulate in catecholamine neurons of substantia nigra (SN) and locus coeruleus (LC), which are preferentially lost in Parkinson's disease (PD). The occurrence of NM in these neurons has been suggested to play a role in their vulnerability in PD. We have found that NM can play either a protective or toxic role in PD depending on cellular context. In aging we have observed that concentrations of NM linearly increase to attain high values like 3-4 mg/g wet tissue in human SN and LC between 80 and 90 years and the loss of catecholamine neurons in PD induces a > 40% decrease of NM concentration. This decrease of NM content of SN can be demonstrated by MRI and this method for imaging neuronal loss is becoming a new method to confirm PD diagnosis. Purified NM is insoluble and we have found it has a structure with the following components: melanic (56%), dolichol lipids(25%), cross-beta-sheet proteins (12%) inorganics(7%). NM is contained in special autolysosomes with lipid bodies and proteins. We have demonstrated that synthesis of NM depends on cytosolic concentration of catecholamines, not accumulated in synaptic vesicles, which is controlled by vesicular monoamine transporter 2 (VMAT2) expression. We have suggested that in cytosol catecholamine adducts with cross-

beta-sheet proteins are formed, then the catecholic moiety is oxidized to produce protein-melanin that is accumulated in autolysosomes, where it is cleaved by proteases and reacts with dolichols to form NM. Synthesis of NM is a protective mechanism because the melanic component is formed by removing reactive quinones whose accumulation would produce neurotoxicity. Based on our culture and tissue studies by others it can be concluded that neurons with high NM content have high cytosolic catecholamine level, high vulnerability and low expression of VMAT2 and vice versa. NM can chelate redox/toxic metals and form stable non toxic complexes so that it is neuroprotective also in this way. Metals accumulated by NM include the highly toxic Pb and Hg, in addition to Fe, Zn, Al, Cr and Mo. NM can play also toxic role in PD. Extracellular NM released by dying neurons of SN can activate microglia with production of H2O2, NO and pro-inflammatory factors then causing further neurodegeneration, with release of NM, microglia activation and so on. This generates a vicious cycle of neuroinflammation/neurodegeneration contributing to progression of PD. The major histocompatibility class I complex (MHC-I) is accumulated in NM-containing organelles of the SN and LC neurons which degenerate in PD. MHC-I can bind antigens derived from foreign proteins, presenting them on neuronal membrane. Then CD8+ cytotoxic T-cells, observed in proximity of MHC-I presenting neurons of SN and LC in PD subjects, can target these neurons.

Relationship of striatal presynaptic dopamine to midbrain neuromelanin in a nonhuman primate model of maternal immune activation

Jason Smucny[1], Tyler Lesh[1], Melissa Bauman[1], Abhijit Chaudhari[1], Shuai Chen[1], Ana-Maria Iosif[1], A. Kimberly McAllister[1], Douglas Rowland[1], Martin Styner[2], Roza Vlasova[2], Guobao Wang[1], Cameron Carter[1]
[1]University of California, Davis, [2]University of North Carolina, Chapel Hill

Background: Maternal infection during pregnancy is a significant risk factor for future mental disorders (including schizophrenia) in offspring. This phenomenon is studied translationally using maternal immune activation (MIA) models, including a unique non-human primate (NHP) MIA model developed at our Center. Dopamine (DA) dysregulation is a feature of some MIA model systems as well as psychotic disorders. We previously reported increased striatal DA synthesis capacity in a pilot pre-adolescent MIA NHP cohort. Neuromelanin (NM) is a non-invasive MRI based measure that is considered to index activity in the DA system. There is a need, however, for additional data validating its relationship to previously established measures of DA activity. In the present study, we report data on the relationship between [18F] fluoro-l-m-tyrosine (FMT) PET measures in the striatum and substantia nigra (SN) NM levels in MIA NHP's. Methods: This study included 13 MIA-treated animals and 14 controls. FMT PET data were analyzed at 15, 26, 38, and 45 months of age in the bilateral caudate, putamen and nucleus accumbens. The Patlak reference tissue model (cerebellar reference) was used to extract DA signal. 3T MRI was used to measure NM levels in the SN at 24, 36, and 45 months of age. The SN ROI was defined by manual tracing based on location and relative NM signal. Results: FMT PET revealed a significant increase in caudate presynaptic DA at 26 months of age in the MIA group vs. controls ($\rho = .025$) but not at other time points. A qualitatively similar the pattern of results was observed in the nucleus accumbens. A significant positive correlation was observed between SN NM and presynaptic DA in the nucleus accumbens in the FMT-treated group ($r = .64$, $\rho = 0.018$) and across all animals ($r = .39$, $\rho = .046$). Conclusions: These results suggest that MIA is associated with a transient increase in caudate presynaptic DA expression during pre-adolescence in NHPs, further confirming the translational relevance of the model. The significant association between NM and PET-measured DA in the

accumbens provides additional validation for using MRI-derived NM signal as a noninvasive proxy marker for DAergic activity in the brain.

MRI Neuromelanin accumulation in patients with treatment-resistant schizophrenia: A cross-sectional pilot study

Fumihiko Ueno[1], Yusuke Iwata[1], Shinichiro Nakajima, Sofia Chavez, Fernando Caravaggio, Guillermo Horga, Clifford Cassidy[2], Edgardo Torres-Carmona, Jianmeng Song, Vincenzo de Luca, Sakiko Tsugawa, Shiori Honda, Sho Moriguchi, Yoshihiro Noda, Mahavir Agarwa
[1]Centre for Addiction and Mental Health, [2]University of Ottawa

Background: Neuromelanin (NM) is a product of monoamine metabolism including dopamine (DA). NM-sensitive MRI sequences allow in vivo quantification of NM levels in the substantia nigra (SN). NM-MRI signal is thought to serve as a biomarker for SN dopamine neuron integrity, and in turn, striatal DA functioning. Increased striatal DA synthesis has been associated with response to first-line antipsychotics (FLR) in patients with schizophrenia, while normal striatal DA synthesis has been associated with treatment-resistant schizophrenia (TRS). As such, we hypothesised that FLR patients would show increased SN-NM levels and TRS would show normal SN-NM levels. Methods: We enrolled TRS patients that had not responded to at least two antipsychotics and were receiving clozapine at the time of the study, patients with FLR, and healthy controls (HCs). SN-NM levels were measured using 3T MRI. Contrast-to-noise (CNR) was calculated as the relative signal intensity difference between SN and crus-cerebri. SN-CNR were compared between groups controlling for age and sex. Correlation coefficients were estimated between CNR and Positive and Negative Symptom Scale (PANSS) scores. Results: 44 participants (TRS, n=13; FLR, n=11; HCs, n=20) completed the study. Overall group differences were found in SN-CNR ($p<0.01$, $\eta2=0.22$). Specifically, FLR but not TRS showed higher SN-CNR compared to HCs ($p<0.01$, Cohen's $d=1.34$). SN-CNR in the patient samples showed no associations with PANSS score. Conclusion: Our results suggest that SN-NM levels are elevated in FLR patients and similar to controls in TRS patients. Longitudinal studies are required to establish if SN-NM levels are a suitable biomarker to predict treatment response in schizophrenia.

Parallel Session 3 – The dopamine D2 receptor: From molecules to behavior

Characterization of a Novel Allelic Variant of the Human Dopamine D2 Receptor

Kim Neve[1], Dayana Rodriguez-Contreras[2], Brooks Robinson[2], Alec Condon[2], Michelle Kielhold[2], Naeem Asad[3], Timothy Dore[3], John Williams[4], Ujwal Shinde[2]
[1]VA Portland Health Care System, [2]Oregon Health & Science University, [3]New York University Abu Dhabi, [4]Oregon Health and Science University

BACKGROUND AND AIM: A novel DRD2 variant has been identified encoding a D2 receptor (D2-Mut) with an amino acid change in the third cytoplasmic loop. The purpose of this study is to characterize the effect of the sequence change on D2 receptor function. METHODS: We characterized the novel variant in HEK 293 cells using radioligand binding and several bioluminescence resonance energy transfer (BRET) assays, in mouse brain slices using electrophysiological methods, and in silico with homology modeling and molecular dynamics simulations. RESULTS: In HEK293 cells, the D2-Mut receptor density is about 35-40% of D2-WT. We assessed arrestin recruitment, G protein activation, and stimulation of cyclic AMP

accumulation by D2-Mut. Maximal arrestin recruitment by D2-Mut was ~half that of D2-WT, even when expressed at similar levels, and was characterized by a faster decay, compared to D2-WT, and by almost complete dependence on overexpression of GRK2. In contrast, quinpirole dose-response curves for activation of Gαi by D2-Mut were left-shifted 5- to 7-fold compared to D2-WT, indicative of more efficient G protein activation. Basal activation of G protein in cells expressing D2-Mut was increased by 30% of the maximal response to D2-WT, consistent with enhanced constitutive (unliganded) activity. We also observed left-shifted dose-response curves and increased basal activity for inhibition of forskolin-stimulated cyclic AMP accumulation by D2-Mut. After AAV-mediated expression in dopamine neurons of D2 autoreceptor knockout mice, both variants mediated synaptic currents in response to release of endogenous dopamine, whether spontaneous or elicited by electrical stimulation, and in response to iontophoretic dopamine, but time-to-peak was slower and peak half-width was greater for D2-Mut. Uncaging of photoactivated sulpiride (CyHQ-sulpiride) produced an apparent inward current of about 10 pA in cells expressing D2-WT, consistent with inhibition of a tonic GIRK current. CyHQ-sulpiride photolysis produced a much larger inhibition of 60-70 pA in cells expressing D2-Mut. To determine whether the sulpiride response reflected inverse agonism of constitutive activity or antagonism of endogenous dopamine, slices were treated with reserpine 1 hr prior to recording. Reserpine-induced dopamine depletion abolished the response to sulpiride in cells expressing D2-WT and greatly decreased the response in cells expressing D2-Mut, consistent with our interpretation that D2-Mut exhibits modestly more constitutive activity and substantially greater sensitivity to dopamine than D2-WT. Molecular dynamics simulations of inactive- and active-state homology models of the two variants suggest that a highly conserved TM3-6 ionic lock is spontaneously disrupted in D2-Mut. CONCLUSIONS: D2-Mut is a G protein-biased receptor with enhanced constitutive activity due to disruption of a TM3-6 ionic lock.

Arrestin recruitment to dopamine D2 receptor mediates locomotion but not incentive motivation

Jonathan Javitch[1]
[1]Columbia University and New York State Psychiatric Institute

BACKGROUND AND AIM: The dopamine (DA) D2 receptor (D2R) is an important target for the treatment of neuropsychiatric disorders such as schizophrenia and Parkinson's disease. However, the development of improved therapeutic strategies has been hampered by our incomplete understanding of this receptor's downstream signaling processes in vivo and how these relate to the desired and undesired effects of drugs. D2R is a G protein-coupled receptor (GPCR) that activates G protein-dependent as well as non-canonical arrestin-dependent signaling pathways. Whether these effector pathways act alone or in concert to facilitate specific D2R-dependent behaviors is unclear. METHODS: We created mutant D2Rs that are extremely biased toward either arrestin recruitment or G protein activation, to the nearly complete exclusion of the other pathway. We evaluated these constructs in a heterologous expression system and then using viral expression in "indirect pathway" medium spiny neurons (iMSNs) in the ventral striatum of D2R KO and wild type mice. We confirmed absence of G protein activation using GTPgS binding assays in striatal homogenates and evaluated basal and cocaine-induced locomotion as well as incentive motivation in a progressive ratio task. RESULTS: We developed a D2R mutant that recruits arrestin but is devoid of G protein activity. When expressed virally in iMSNs in the ventral striatum of D2R knockout mice, this mutant restored basal locomotor activity and cocaine-induced locomotor activity in a manner indistinguishable from wild-type D2R, indicating that arrestin recruitment

can drive locomotion in the absence of D2R-mediated G protein signaling. In contrast, incentive motivation was enhanced only by wild-type D2R, signifying a dissociation in the mechanisms that underlie distinct D2R-dependent behaviors, and opening the door to more targeted therapeutics. We also developed two constructs that show extreme bias for G protein signaling in HEK293 cells. When expressed virally in iMSNs using the identical methodology as the arrestin-biased D2R discussed above, we find that expression is extremely poor. This raises the possibility that in neurons arrestin interaction might be important for D2R biosynthesis, surface expression, and/or stability, although we cannot rule out deleterious effects of these mutations unrelated to arrestin interaction. CONCLUSIONS: The search for biased ligands postulates that these effector pathways might be differentially important in the various functions of the receptor. This hypothesis cannot yet be properly interrogated with existing ligands, as they are not sufficiently biased or circuit selective. This has motivated us to pursue the creation of D2R constructs that are extremely biased in their signaling properties, which has begun to support the idea that differential engagement of the two pathways can indeed lead to different behaviors, providing promise but also challenges to future drug discovery in this arena.

Cocaine-Induced Changes in D2 Receptor Signaling

Christopher Ford[1]
[1]University of Colorado Anschutz Medical Campus

BACKGROUND AND AIMS: Dopamine input to the dorsal and ventral striatum originates from separate populations of midbrain neurons. Despite differences in afferent inputs and behavioral output, little is known about how dopamine release is encoded by dopamine receptors on medium spiny neurons (MSNs) across striatal subregions. Drugs of abuse including cocaine are known to induce long-term changes in striatal and acumbal circuitry, however it remains unknown how exposure to drugs of abuse alter the properties of dopamine receptors themselves. The goal of this talk will be describe changes in D2-receptor function that are induced by cocaine. METHODS: To assess the regional difference in D2R transmission, we virally overexpressed G protein-coupled inward rectifying potassium (GIRK2) channels in medium spiny neurons (MSNs) to provide and electrophysiological read out of D2-receptor activation. After allowing three weeks to allow expression of GIRK2 in D2-MSNs male and female mice were given a series of non-contingent injections of cocaine and the resulting changes in D2-MSN physiology were examined. RESULTS: We found that 7-day exposure to cocaine selectively reduced the sensitivity of D2-receptors in the nucleus accumbens which resulted from an alteration in the local expression of different G-protein subunits in D2-MSNs. CONCLUSION: These results identify that exposure to cocaine can selectively regulate the sensitivity of D2-receptors in distinct striatal regions. The long-term changes by which dopamine signals are encoded in nigrostriatal and mesolimbic circuits may in turn influence underlying behaviors that are regulated by these circuits.

New functional maps of cortical dopamine receptors

Martin Beaulieu[1]
[1]University of Toronto

BACKGROUND AND AIM: The D2 dopamine receptor remains a major target for symptom remediation in schizophrenia and Parkinson's disease. While functions of striatal D2 receptors have been studied

extensively, much less is known about D2 receptor functions in several cortical areas. However, the study of cortical D2 receptors expression and functions has for the most part been restricted to a subset of pyramidal neurons and interneurons (e.g. parvalbumine positive) of the pre frontal cortex where D2 regulated local circuits are believed to contribute to the regulation of emotional and cognitive functions. Our objective was to develop new approaches allowing the characterization of cortical dopamine receptor expression and functions in a circuit defined manner. METHODS: To map dopamine receptor expression, we used combinations of transgenic reporter systems, recombinase activated viral vectors, quantitative translatome analysis, and high sensitivity in situ hybridization to identify D2 receptor expressing cells and establish a map of their respective projections. To characterize dopamine receptor functions, we developed CRISPR/Cas9 based approaches to manipulate dopamine receptor expression with neural circuit selectivity. RESULTS: Our results identified previously uncharacterized clusters of D2 expressing neurons in limbic and sensory regions of the adult mouse brain cortex. Characterization of these clusters by translatome analysis and cell type specific labeling revealed highly heterogeneous expression of D2 receptors in principal neurons and various populations of interneurons across cortical areas. Circuit defined characterization of D2 receptor functions using CRISPR/Cas9 allowed identifying the contribution of D2 receptors in specific pyramidal neurons to emotional regulation, locomotion and drug responsiveness. CONCLUSIONS: These results pave the way for a thorough re-examination of cortical D2 receptor functions, which could provide information about neuronal circuits involved in psychotic and mood disorders.

Parallel Session 4 – Mechanisms controlling the excitability of midbrain dopaminergic neurons

A new hypothesis for slow pacemaking of nigral dopamine neurons

Vincent Seutin[1]
[1]Liège University

BACKGROUND AND AIM: Slow (0.5 to 4 Hz) endogenous pacemaking is a hallmark of midbrain dopaminergic (DA) neurons when they are recorded in tissue slices. This activity is believed to ensure an adequate sensitivity of postsynaptic DA receptors in target areas. Despite many years of investigation, the exact mechanism of this pacemaking remains unknown. For example, superfusion of blockers of the main voltage-gated and voltage independent channels expressed in these neurons fails to abolish pacemaking. We tested the hypothesis that smaller conductance pores, called gating pores, could underlie the tiny current needed for pacemaking. METHODS: We used a blocker of gating pore currents called 1-(2,4-xylyl)guanidinium (XG) and tested its effect on the excitability of DA neurons using patch clamp recordings in slices from juvenile rats and mice (collaboration with Pr. J. Roeper) and extracellular recordings in slices from adult rats. RESULTS: We found that XG inhibits the firing of DA neurons both in rat and mouse DA neurons. In adult rat neurons, its IC50 (concentration needed to decrease the firing rate by 50%) was 485 microM. This effect appeared to be due to a direct inhibition of the pacemaking current because XG did not increase the conductance of DA neurons and did not hyperpolarize them. In addition, firing could be reinstated in the presence of XG by injecting small amounts of positive current (10-30 pA) and no action potential parameter was affected by the drug. Finally, the effect of XG appeared to be specific of slow pacemaking because the firing of reticulata GABAergic neurons, which are fast pacemakers, was unaffected by the drug. CONCLUSIONS: XG appears to be a selective blocker of the pacemaker current of DA neurons. Further electrophysiological and molecular experiments are being performed to identify its exact target.

Selective gain control of dopamine substantia nigra neurons by Cav1.3 channels - a feasible target for activity-dependent neuroprotection in PD

Josef Shin
[1]*Frankfurt University*

Morphological and biophysical determinants of action potential shape and pacemaking in dopaminergic neurons

Jean-Marc Goaillard[1]
[1]*Université d'Aix-Marseille*

Background and Aim. Within a given population of neurons, electrical activity always displays a significant level of variation from cell to cell. What determines these cell-to-cell variations in pattern of activity or, more generally, in electrophysiological parameters ? Are the variations in morphological properties (dendritic complexity, dendritic length, axon morphology) or in biophysical properties (ion channel density) mainly responsible for the cell-to-cell variations in electrical phenotype ? We addressed these questions on midbrain dopaminergic neurons, trying to unravel the relative impact of morphology and voltage-dependent ion channels on both pacemaking and action potential shape. Methods We used electrophysiological recordings of rat substantia nigra pars compacta dopaminergic neurons to define the level of cell-to-cell variations in pacemaking and action potential shape. Neurons were then reconstructed and full dendritic morphology and axon initial segment (AIS) geometry were obtained. Realistic Hodgkin-Huxley computational models were then built based on the full morphology of the recorded neurons. Results Using this combination of methods, we showed in a first study (Moubarak et al., 2019) that pacemaking in rat neurons seems to be mainly controlled by the somato-dendritic compartment, due to the expression of a relatively high density of voltage-gated sodium channels in this compartment. Our results also suggested that AIS geometry has a limited influence on pacemaking in this type of neurons, unlike what was suggested by results obtained in vivo from mouse neurons (Meza et al., 2018). In a second study, we made the interesting observation that action potential shape was strongly correlated with dendritic topology: axon-bearing and non-axon bearing dendrites were found to display oppositive correlations with the duration of somatic action potential. Using modelling and dendrotomy experiments, we showed that the influence of dendrites on action potential shape was determined by their contents in voltage-gated sodium channels, suggesting that sodium channel expression might not be strictly homogeneous in dopaminergic neurons. Conclusions. Mibdrain dopaminergic neurons, while displaying a very long and highly branched axon, are considered to present a fairly simple dendritic arborization. In the work presented here, we show that, despite this relative simplicity, cell-to-cell variations in dendritic complexity have a very strong influence on neuronal output, due in particular to the expression of voltage-gated sodium channels in the somato-dendritic compartment. These studies highlight the impact of morphological variations on neuronal output in pacemaking neurons.

Mechanisms of GABA-A receptor mediated control of midbrain dopaminergic neuron axons

Paul Kramer
[1]*NINDS/NIH*

We tested how GABA-A receptors influence the excitability of dopaminergic neuron axons and the release of dopamine onto targeted cells in the dorsal striatum. To do this, we performed direct whole-cell and perforated-patch recordings from the cut ends of the main unbranching dopaminergic neuron axons and from axonal projections within the dorsal striatum. We found that brief application of GABA to axons led to depolarization of the subthreshold membrane potential but also decreased the peak amplitude of propagating axonal action potentials. In addition, GABA-A receptor activation inhibited the spread of action potentials throughout the axonal arbor which resulted in reduction of striatal dopamine release. The mechanism of axonal inhibition involves shunting in combination with inactivation of voltage-gated sodium channels. Lastly, we tested the effect of diazepam, a broad-spectrum positive allosteric modulator of GABA-A receptors. Diazepam enhanced axonal GABA-A receptor currents which led to the reduction of axonal input resistance and inhibition of striatal dopamine release. Therefore, our data provide definitive evidence for the existence of GABA-A receptors on the axons of dopaminergic neuron. Furthermore, we reveal the mechanisms of GABA-A receptor modulation of dopamine release and provide new insight into the role of axonal GABA-A receptors in the actions of benzodiazepines in striatum.

Parallel Session 5 – LRRK2 and GTPase activity

Structural Bases for Nucleotide-dependent Conformational Dynamics in the G-domain of LRRK2

Quyen Hoang[1]
[1]*Indiana University School of Medicine*

BACKGROUND AND AIM: We recently showed that the R1441H mutation perturbs the hydrolytic conversion of GTP to GDP, thereby prolonging the GTP-bound "on" state of ROC. Subsequently, we showed that ROC undergoes nucleotide-dependent dimer-monomer conformational changes, wherein GTP-binding drives dimeric ROCext disassembly into monomers and GDP-binding oppositely shifts the equilibrium back towards the dimeric conformation, and that all the PD-associated mutations at Arg-1441, as well as N1437H, impair this conformational dynamic. However, the mechanism(s) by which this impairment occurs is unclear since position 1441 is distal to the regions directly involved in GTPase activity, including the nucleotide-binding site, active-site, and the switch regions. The PD-protective mutant R1398H, on the other hand, resides in the active-site of ROC; however, its effects on the structure and function are not known. Our aims are to determine the mechanism(s) of nucleotide-dependent conformational changes that occur in the ROC domain of LRRK2. METHODS: X-ray crystallography, molecular dynamic simulation, protein biochemistry. RESULTS: We have determined high-resolution crystal structures of ROC revealing the structure and interactions that occur at residue R1441 that regulate the dimer-monomer conformational dynamics, and providing insights into the mechanisms by which the PD-associated mutations R1441G/C/H impair this dynamic process. We have also determined the structure of the R1398H mutant, which along with molecular dynamic simulation and biochemical data, supports that residue R1398 regulates the conversion of ROC dimers to monomers upon binding GTP. CONCLUSIONS: Residue R1441 stabilizes the ROC-ROC dimeric interface and residue R1398 triggers conformational changes upon binding GTP.

Neurodegenerative Mechanisms of LRRK2 Mutations in Parkinson's Disease: Role of GTPase Activity and Dimerization

Darren Moore[1]
[1]Van Andel Institute

BACKGROUND AND AIMS: Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene cause late-onset, autosomal dominant familial Parkinson's disease (PD), whereas common LRRK2 variants contribute to the risk of sproadic PD. LRRK2 is a member of the ROCO protein family, characterized by multiple domains, including a Ras-of-Complex (Roc) GTPase domain and a protein kinase domain separated by a C-terminal-of-Roc (COR) domain. Familial LRRK2 mutations are now known to commonly enhance kinase activity and substrate phosphorylation in cells, either directly by altering the kinase activation loop (i.e. G2019S, I2020T) or indirectly by impairing GTP hydrolysis (i.e. R1441C/G/H, Y1699C). Familial LRRK2 mutations also commonly induce neuronal toxicity in primary culture models via a kinase-dependent mechanism yet the contribution of the GTPase domain to toxicity is poorly understood. Accordingly, our studies have focused on exploring the contribution of i) GTPase activity and ii) dimerization via the Roc-COR domain, to the neurotoxic effects of LRRK2 mutations. METHODS: To dissect the role of GTPase activity in regulating neurodegeneration induced by human G2019S LRRK2 in the rat brain, we employ viral-mediated gene transfer methodology using recombinant human adenovirus serotype 5 (Ad5) vectors to induce expression of full-length human LRRK2 variants within the rat nigrostriatal pathway. We also combine structural homology modeling, mutational analysis, biochemical and cell biological approaches to explore the mechanisms of LRRK2 dimerization in cells. RESULTS: We find that the Ad5-mediated expression of G2019S LRRK2 in the rat brain induces the progressive degeneration of substantia nigra dopaminergic neurons. Intriguingly, we find that introduction of mutations that enhance GTP hydrolysis (R1398L) or impair GDP/GTP-binding (T1348N) significantly attenuate neurodegeneration induced by G2019S LRRK2 in rats. We also probe the mechanisms of LRRK2 dimerization via the Roc-COR tandem domain. Our data provide biochemical support for key interactions between Roc domains within the predicted dimer interface, as well as between Roc and COR domains within individual monomers. Importantly, disrupting these interaction interfaces is sufficient to impair the dimerization and enzymatic activities of LRRK2. CONCLUSIONS: Our study provides important insight into the pathogenic mechanisms underlying familial LRRK2 mutations, and nominates GTPase modulation and interference with Roc-COR-mediated dimerization as potential therapeutic strategies for attenuating LRRK2 activity in PD.

LRRK2 and Dopaminergic Neurotransmission

Hui Zhang[1]
[1]Thomas Jefferson University

BACKGROUND AND AIM: Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the most frequent causes of familial Parkinson's disease (PD), and contribute to idiopathic cases. Given its strong genetic links, LRRK2 represents a compelling therapeutic target for PD. However, the mechanisms that regulate LRRK2 function and the pathogenic effects of mutations remain unclear. The LRRK2 protein includes two enzymatic domains: a ROC (Ras of Complex) GTPase domain and a kinase domain. Disease causing mutations are found in both enzymatic domains, indicating their importance in pathogenesis.

While studies have mainly focused on LRRK2 kinase activity, attention on the GTPase activity is limited. Therefore, in this study, we have focused on examining whether the dopaminergic neurotransmission defects in LRRK2 mouse models are kinase dependent or not. METHODS: We use transgenic mouse models with mutations either in the kinase domain (G2019S) or in the GTPase domain (R1441G) and LRRK2 kinase inhibitors to examine whether the deficits are kinase dependent or not. We employ fast-scan cyclic voltammetry to examine dopamine (DA) release in the dorsal striatum and patch clamp to examine DA neuron firing in the substantia nigra pars compacta (SNpc) in mouse brain slices. RESULTS: Both R1441G and G2019 transgenic mice exhibit decreased DA release and synaptic vesicle recycling and LRRK2 kinase inhibitors can alleviate the deficits. Dopamine D2 receptors are prototypical autoreceptors that control the firing rate of pacemaking SN DA neurons. Decreased D2R mediated autoinhibition can lead to increased excitability of DA neurons and result in excitotoxicity and neurite degeneration. We find that while D2R-mediated inhibition of SN DA neuron pacemaker activity is pronounced during maturation in wild-type mice, the maturation of the D2R response is absent in R1441G transgenic mice but not in G2019S transgenic mice. Consistently, LRRK2 kinase inhibitors cannot alleviate the defects of D2R modulation in R1441G transgenic mice. CONCLUSIONS: Our study suggest that LRRK2 kinase activity and GTPase activity play different roles in dopaminergic neurotransmission and PD pathogenesis. Developing GTPase-modulation-based approach will be a new and important strategy for the treatment of LRRK2 PD patients.

Identification of LRRK2 GTP Binding Inhibitors towards PD Therapeutics

Wanli Smith[1]
[1]Johns Hopkins University School of Medicine

Background and Aim: Mutations in the leucine-rich repeat kinase-2 (LRRK2) gene cause autosomal-dominant Parkinson's disease (PD) and contribute to sporadic PD. LRRK2 contains Guanosine-5'-triphosphate (GTP) binding, GTPase and kinase activities that have been implicated in the neuronal degeneration of PD pathogenesis, making LRRK2, a potential drug target to develop LRRK2 inhibitors for PD intervention. My presentation covers the research findings from our group on identification and characterization of the LRRK2 GTP binding inhibitors. Methods and Results: Through a combination of computer-aided drug design (CADD) and LRRK2 bio-functional screens, we have identified a series of novel compounds that can reduce LRRK2 GTP binding and inhibit LRRK2 kinase activity in vitro and in vivo. Further characterization studies demonstrate that LRRK2 GTP binding inhibitors can protect against PD-linked mutant LRRK2-induced neuronal degeneration, altering B cell functions and reducing inflammation, and promoting protein ubiquitination and Lewy body-like inclusion formation. Conclusions: These LRRK2 GTP binding inhibitors provide useful tools for further studying the LRRK2 functions and for developing potential therapeutics for LRRK2-linked PD intervention.

Parallel Session 6 – The intriguing axonal connectivity of dopamine neurons

Exploring the synaptic and non-synaptic connectivity of dopamine neurons

Louis-Eric Trudeau[1]
[1]Université de Montréal

Dopamine and other neuromodulatory neurons in the brain appear to be quite distinct in their connectivity compared to the larger contingent of more classic, fast-signaling glutamate and GABA neurons. Dopamine neurons are endowed with a particularly broad and arborized axonal domain containing an extremely large number of release sites. The discovery in the 1980s that most axonal varicosities established by these neurons do not display a classical synaptic structure is a long-standing mystery. We have been recently exploring the topography of dopaminergic release sites using a co-culture system of postnatal mouse dopamine neurons together with striatal medium spiny neurons. We find that contrarily to cortical or striatal neurons, dopamine neurons have a surprising propensity to establish axonal varicosities that mostly ignore target cells. These axonal varicosities contain classical vesicular proteins such as synaptotagmin 1 and VMAT2. Only a very small subset of dopaminergic terminals are in close proximity to postsynaptic domains containing dopamine D2 receptors or postsynaptic organizers such as PSD95 or gephyrin. We find this small subset of "synaptic" release sites can be distinguished from the larger subset of "non-synaptic" terminals by their differential expression of active zone proteins. In stark contrast, the vast majority of release sites established by cortical or striatal neurons establish tight links with postsynaptic domains. Whether synaptic or not in structure, we find that in this co-culture system, most dopaminergic axonal varicosities appear to be functional as revealed by the activity-dependent uptake and release of synaptic vesicle recycling markers. A better understanding of the unique connectivity of neuromodulatory neurons is bound to provide new insights into their normal physiological roles and their impairment and vulnerability is diseases such as Parkinson's, drug abuse and schizophrenia.

Striatal mechanisms for fast dopamine signaling

Pascal Kaeser[1]
[1]Harvard Medical School

Dopamine is a neuromodulator that codes information on various time scales. I will discuss recent progress on the identification of fast release mechanisms for dopamine in the mouse striatum, evaluate the molecular machinery that controls fast release, and present data on triggering mechanisms of dopamine release that are independent of action potentials ascending from the dopamine neuron somata in the midbrain. These mechanisms may account for fast dopamine coding during dopamine-dependent behavioral tasks, and may explain dopamine neuron-mediated functions independent of somatic action potential firing. In the long-term, our work will allow for a better understanding of the mechanisms and time scales of dopamine coding in health and disease.

Delineating the molecular architecture of dopaminergic release sites by super-resolution microscopy

Ulrik Gether[1]
[1]University of Copenhagen

Each dopamine (DA) neuron possesses numerous presynaptic release sites consisting of a specific repertoire of molecular components of which some are unique to DA or monoaminergic neurons while others make up generalized synaptic features. However, due to the small size of DA release sites, we know little about the nanoscale organization of these components and the biological and pathobiological implications. To address this, we employ single-molecule super-resolution microscopy

techniques, such as direct stochastic optical reconstruction microscopy (dSTORM), that represent unique tools to investigate the molecular architecture of subcellular structures. Our experiments have revealed e.g. how the DA transporter (DAT) can move in and out of discrete cholesterol-enriched nanodomains within the presynaptic release site, and that this is regulated by neuronal activity through a calcium-dependent mechanism with DAT assuming a distinct functional configuration when present in the nanodomains. The data show how super-resolution microscopy in quantitative manner can visualize architectural changes at DA release sites that might be of critical for DA function, DA pathology and drug responses.

Novel roles for dynamic VGLUT expression in dopamine neurons from flies to humans

Zachary Freyberg[1]
[1]University of Pittsburgh

The ability of presynaptic dopamine (DA) terminals to tune neurotransmitter release to meet the demands of neuronal activity is critical to neurotransmission. Although vesicle content was assumed to be static, we recently showed that cell depolarization increases synaptic vesicle DA content prior to release via vesicular hyperacidification. This depolarization-induced hyperacidification is mediated by the vesicular glutamate transporter (VGLUT) in both fly and mouse. In response to depolarization, DA vesicles utilize VGLUT to dynamically increase the vesicular pH gradient, thereby increasing dopamine vesicle content. We therefore hypothesized that the expression of VGLUT in DA neurons may also be dynamic, particularly in response to conditions that alter synaptic DA levels. To test this, we developed a novel intersectional genetic reporter of VGLUT expression specifically in DA neurons in our Drosophila model. We found that the expression of VGLUT in presynaptic DA neurons is indeed highly dynamic, particularly in response to changes in synaptic DA levels caused by amphetamine or reserpine as well as in response to aging. In rodents, we also found that in the rotenone Parkinson's disease model, rotenone exposure not only caused progressive DA neuron degeneration, but also increased VGLUT2 expression in surviving DA neurons. This is consistent with findings in both fly and mouse models suggesting a role for VGLUTs in DA neuron resilience and plasticity. Furthermore, we find striking sexual dimorphism in VGLUT expression in DA neurons with females expressing significantly more VGLUT than males - an effect conserved across flies, rodents and humans. Overall, our data provide new functional relevance to dopamine/glutamate co-transmission in both healthy and DA neuron disease states.

Parallel Session 7 – Dopamine D2/3 receptors and responses to rewards: More complicated than we thought

Extra-striatal D2/3 receptor availability in youth at risk for addiction and addiction-related disorders

Marco Leyton[1]
[1]McGill University

Background and Aim: The causes of addictions and addiction-related disorders remain poorly understood. However, in laboratory animals, dopaminergic function throughout the prefrontal cortex, limbic system, and upper brainstem has been implicated in behavioural features that influence addiction vulnerability, including poor impulse control and altered sensitivity to rewards and punishments; i.e., externalizing features. These dopamine and externalizing features are both altered by exposure to

environmental stressors. To test these associations in humans, we measured externalizing traits, early life trauma, type-2/3 dopamine receptor (DA2/3R) availability and clinical outcomes in youth who have been followed since birth. Methods: Fifty-eight participants (18.5±0.6yr) were recruited from longitudinal cohorts in Quebec: half had high (high EXT, N=27, 16F/11M) and half had low externalizing traits (low EXT, N=31, 20F/11M). All underwent a 90-min positron emission tomography [18F]fallypride scan and completed the Barratt Impulsiveness Scale (BIS-11), Substance Use Risk Profile Scale (SURPS), and Sensitivity to Punishment (SP) & Sensitivity to Reward (SR) Questionnaire; most completed the Childhood Trauma Questionnaire (CTQ; N=56). Forty-one participants had follow-up interviews two to three years later. The three-factor model was tested using binomial logistic regression. Results: The high vs. low EXT participants reported elevated substance use, BIS-11, and SR and SURPS Impulsivity scores, had a greater prevalence of psychiatric disorders, and exhibited higher [18F]fallypride binding potential (BPND) values in prefrontal, limbic and paralimbic regions, even when controlling for substance use. Across all participants, low midbrain BPND values were associated with low SP scores and high SR / SP ratios. Most strikingly, the combination of low midbrain BPND values, high CTQ scores and high EXT scores predicted with high accuracy (>90%) and statistical robustness ($p = 0.000024$) lifetime histories of substance use disorders and their commonly comorbid conditions. This same three-factor model (BPND, CTQ, EXT) continued to predict disorders two to three years later. Conclusion: A combination of EXT traits, early life adversity, and low dopamine autoreceptors might increase risk for diverse early onset commonly comorbid disorders. The data reported here raise the possibility that these features can predict susceptibility prospectively.

Brain levels of Dopamine D3 receptors are linked with a genetic variation in the endocannabinoid-degrading enzyme FAAH

Laura Best[1], Isabelle Boileau[1]
[1]*University of Toronto*

Background: The endocannabinoid and dopaminergic systems have been implicated in addictions. Our changing studies have independently shown that greater dopaminergic transmission at the D3 dopamine receptor as well as elevated endocannabinoid singling may contribute to motivation to use drugs in persons with substance use disorders. Methods: Binding of the dopamine D3 preferring probe [C-11]-(+)-PHNO was measured with positron emission tomography (PET) in 79 human subjects genotyped for the <I>FAAH</I> C385A polymorphism (36/79 AC+AA). Autoradiography with [H-3]-(+)-PHNO and in situ hybridization with a D3-specific S-35 riboprobe were carried out in 30 knock-in mice with the <I>FAAH</I> C385A polymorphism (20/30 AC+AA). Results: We found that the <I>FAAH</I> genetic variant C385A was associated with significantly higher (+)-PHNO binding in both humans and in knock-in mice and this effect was restricted to D3 selective regions (limbic striatum, globus pallidus, and ventral pallidum (9-14%; $p < 0.04$) in humans and Islands of Calleja (28%; $p = 0.06$) in mice). In situ hybridization with a D3-specific S-35 riboprobe in <I>FAAH</I> knock-in C385A mice confirmed significantly increased D3 receptor mRNA across examined regions (7-44%; $p < 0.02$). Conclusion: The association of reduced FAAH function with higher dopamine D3 receptors in human and mouse brain provide a mechanistic link between two brain systems that have been implicated in addiction-risk. This may explain the greater vulnerability for addiction in individuals with C385A genetic variant and by extension, suggest that a D3 antagonism strategy in substance use disorders should consider <I>FAAH</I> C385A polymorphism.

Regionally-specific Dopamine measures predict impulsivity and attentional control in healthy humans

David Zald[1]
[1]Vanderbilt University

Humans demonstrate substantial regionally-specific individual differences in features of the dopamine system, some of which may be directly relevant to core differences in personality and cognitive abilities. In this talk, I will review data from PET studies using [18F]fallypride to assess midbrain, striatal, and cortical D2/D3 receptor availability in healthy individuals and in patients with Parkinson's disease with an emphasis on associations with self-reported impulsivity and impulse control problems. These findings will be augmented by data from a recent study of striatal dopamine transporter assessed with [18F]-FE-PE2I and amphetamine-induced dopamine release in 44 young and middle-aged adults. To move beyond self-report measures, we also assessed objective measures of attentional control and impulsive responding using the Tests of Vigilance and Attention (TOVA), which is widely used in the neuropsychological assessment of attention deficit hyperactivity disorder (ADHD). Analyses indicate that midbrain and ventral striatal dopaminergic parameters were predictive of TOVA performance and combining different regional measures of dopamine features (midbrain auto receptor levels, ventral or dorsal striatal D2/D3 receptor availability or responsively to amphetamine) further improved prediction in regression analyses. These data highlight how patterns of dopamine variables within different dopamine circuits may influence impulsivity and attention abilities in humans. Specific implications for externalizing psychopathology all be discussed.

Abnormal Dopamine encoding of reward related information in a mouse model of striatal D2 receptor over-expression

Eleanor Simpson[1]
[1]Columbia University

Background and Aim: Imaging studies support the hypothesis that the ethology of schizophrenia involves alterations in dopamine. These changes include an increase in the synthesis and release of dopamine in the striatum including increased occupancy of striatal D2 receptors, the primary target of antipsychotic medications. Aberrant encoding of reward related information within the dopamine system has been hypothesized to underlie at least one symptom from each of the major symptom domains: Reinforcement learning (cognitive), hallucinations (positive) and abolition (negative). In the present study, we aimed to determine if increased stratal D2 receptors might alter the dopamine encoding of reward related information. Methods: We employed a mouse model of increased striatal D2 receptors (D2R-OE mice) that overexposes D2Rs selectively in the postsynaptic output neutrons of the striatum chronically throughout development and adulthood. D2R-OE and control mice were tested in both Pavlovian and operant conditioning paradigms while phasic dopamine release in the ventral striatum (nucleus accumbens, NAc) was monitored in real-time via chronically implanted carbon fibre micro electrodes using fast scan voltammetry. Results: In a Pavlovian conditioning experiment in which two different tones determined the probability of reinforcement, control mice displayed an increase in conditioned approach behavior and increased dopamine release in response to the high probability conditioned cue (75% chance of food reward) compared to the low probability conditioned cue (25% chance of food reward). D2-OE mice failed to show such differential response to these cues at the level

of behavior or dopamine release. In a self-initiated reward-learning operant task with probabilistic reinforcement, we measured NAc dopamine encoding of Reward Prediction Errors. RPEs, reflected by dopamine cell activity, represent the discrepancy between expected and received outcomes and are proposed to serve as a basic process underlying some forms of associative learning. D2R-OE mice displayed altered NAc dopamine encoding of positive, but not negative RPEs. Conclusion: The valence specific deficit in encoding RPEs in D2R-OE mice may be of relevance to the finding that patients with schizophrenia learn normally from negative outcomes, but show a reduced capacity to learn from positive outcomes. Our results suggest that reward learning deficits in patients may result from a valence specific deficit in RPE encoded by NAc dopamine transients.

Parallel Session 8 – Dopamine in the aging brain: Links to cognition, brain integrity, genetics, and lifestyle

Within-person dopamine D2-receptor losses in the aging brain: insights from the Cognition, Brain, and Aging (COBRA) study

Nina Karalija[1]
[1]Umeå University

BACKGROUND AND AIM: The dopamine (DA) system has been described as one of the most age-sensitive systems of the brain. This statement rests on findings from nearly 100 independent imaging studies, demonstrating reduced numbers of DA markers in older, as compared to younger, adults. Several lines of research have demonstrated that DA modulates cognitive processes, and that attenuated DA transmission is accompanied by reduced cognitive performance. Consequently, it has been suggested that DA loss underlies cognitive decline in aging. Past in vivo studies of age-related alterations of the human DA system have typically been performed in cross-sectional settings with limited sample sizes. These features have obstructed studies of individual differences in rates and patterns of DA decline, and failed to address whether within-subject change in DA integrity predicts change in cognitive performance. Furthermore, links among age-related DA decline, other brain changes, and lifestyle and genetic factors remain unknown. METHODS: The Cognition, Brain, and Aging (COBRA) study was designed to cover these gaps in knowledge. Its design includes a multimodal approach, robust sample size, and consecutive testing in 5-year intervals. Specifically, 181 healthy, older adults (ages: 64-48 years at baseline) underwent positron emission tomography with [11C]raclopride for D2/3 receptor assessment, magnetic resonance imaging for evaluation of brain structure and function, cognitive testing, and lifestyle and genetic mapping. RESULTS: Approximately 70% (n=129) of the original sample returned to the 5-year follow-up, during which they repeated all tests. Findings from COBRA demonstrate large individual differences in D2/3 receptor availability at baseline, and in 5-year changes. Notably, the magnitude of striatal D2/3 decline was ~50% of past cross-sectional estimates, suggesting overestimated decline rates in past research. Significant D2/3 receptor reductions were also observed in select extrastriatal regions. Cerebrovascular changes were sizeable, and associated with D2/3 receptor losses across several associative and limbic regions. CONCLUSIONS: These data offer novel insights into long-term intraindividual D2/3 changes in healthy aging.

Dopamine D2-like receptors contribute to neural integrity across different cognitive states

Alireza Salami[1]
[1]Umeå University & Aging Research Center

BACKGROUND AND AIM: Dopamine (DA) is a neuromodulator which plays a critical role in cognitive processes via modulating the blood oxygenation level-dependent (BOLD) signal. Age-related reductions of DA D2/3 receptor (D2/3DR) availability in aging have been linked to age-related alternations in neural responses and concomitant cognitive decline. However, the specific role of D2/3DR in cognition and related neural responses in old age remains inconclusive. RESULTS: Using data from COBRA study, we showed that D2/3DR availability in caudate and hippocampus interrelated and were associated with episodic memory performance. This finding suggests that individuals with higher caudate D2/3DR had higher hippocampal D2/3DR and higher episodic memory. Critically, caudate D2/3DR was positively associated with resting-state functional connectivity between ventral caudate and medial temporal lobe (MTL), which is highly implicated in episodic memory processing. Further path analyses revealed that the association between D2/3DR in caudate and episodic memory was mediated through functional connectivity between ventral caudate and MTL. Using data from the DyNAMiC study, we extended the relationship between connectivity and DA to D1DR system and brain functional architecture. Individuals with the lowest and highest levels of D1DR displayed deviating functional architecture, suggesting that DA may impact the overall repertoire of functional connectivity. Past studies have shown links between DA and working memory and its neural correlates, whereas others have failed to observe such relationships. A possible reason for the discrepancy is differences in task demands, such that a more demanding task with greater prefrontal activations may yield a stronger association with DA. We found a significant positive association of striatal as well as extra-striatal D2/3DRs to BOLD response in the thalamo-striatal-cortical circuits. This finding provides early evidence that cortical D2/3DR is critically important for maintaining a responsive striatal-cortical working memory circuit. Further analyses of individual differences showed that the normal performing subgroup exhibited DA-BOLD association in a load-dependent fashion, with the strongest associations during 3-back condition. In contrast, the low-performing subgroups showed no load-dependent modulation, with strongest associations during 2-back condition. These results suggest a greater need for dopaminergic modulation during a more demanding working memory condition, which varies across subgroups. CONCLUSIONS: Our findings suggest that individual differences in DA availability contribute to differences in the functional architecture of the brain across different mental states. We speculate that age-related decline in DA may lead to disrupted functional integrity and, in turn, cognitive decline in aging.

Aging-related declines in dopamine receptor availability and their links to cognitive changes

Goran Papenberg[1]
[1]Aging Research Center, Karolinska Institute and Stockholm University

BACKGROUND AND AIM: Dopamine (DA) supports molecular mechanisms central for various cognitive functions. Based on cross-sectional data, age-related decline in the DA system has been suggested as a central mechanism underlying cognitive aging. Although the DA-cognition link is one of the most cited mechanisms in cognitive neuroscience, recent large-scale cross-sectional data reported only weak evidence for the correlative triad among aging, dopamine D2-like receptor availability, and cognition. Given that cross-sectional data can be heavily influenced by cohort effects, only longitudinal data can

resolve this discrepancy in the literature. METHODS: We present data on change-change correlations between dopamine (DA) and cognition from the COBRA study in healthy older adults, aged 64-68 years at baseline (n = 129), which assesses DA D2/3 receptor (D2/3DR) availability with [11C]raclopride PET across five years. Changes in cognition were measured for episodic memory, working memory, and perceptual speed. RESULTS: In the baseline sample (n = 181), we previously showed that positive effects of high D2/3DR availability in the hippocampus on episodic memory are confined to carriers of advantageous genotypes of the BDNF and the KIBRA polymorphisms, both implicated in long-term memory. The longitudinal analyses revealed the same pattern: less decline in hippocampal D2/3DR availability was associated with less decline in episodic memory in carriers of advantageous genotypes. This finding is in line with the notion that cognitive functions, such as episodic memory, are polygenic traits influenced by different molecular mechanisms. Overall, we did not observe change-change correlations between D2/3 D2/3DR availability and neither working memory nor speed in the total sample. Critically, however, classifying older adults based on their domain-specific trajectories (improved performance, maintained performance, decline), our results show that changes in D2/3DR availability are related to changes in brain regions relevant to specific cognitive functions. Greater decline in D2/3DR availability in task-related regions of interest (caudate, middle frontal cortex) was correlated with more decline in performance in persons who also experienced impairments in working memory across time. Similarly, greater decline in speed was exclusively correlated with negative putaminal changes D2/3DR availability, again among decliners only. CONCLUSIONS: Our longitudinal analyses support the notion that aging-related changes in the DA system contribute to cognitive aging.

Age-related differences in dopamine D1-receptors across the adult lifespan: insights from The Dopamine, Age, Connectome, and Cognition (DyNAMiC) study

Jarkko Johansson[1]
[1]Umeå university

BACKGROUND AND AIM: Human studies have shown marked age-related differences in dopaminergic D1 systems accompanied by cognitive decline in older individuals. However, most previous observations of age-related differences in dopamine (DA) D1 receptor (D1DR) availability were based on extreme age-group comparisons, which preclude conclusions about the rate and shape of D1DR differences across the adult lifespan. Gene-expression studies suggest that D1DRs exhibit extraordinarily protracted development which may call for a need to differentiate potential phases for D1DR alterations. METHODS: Here, we present preliminary findings from a large-scale population-based study, DyNAMiC. DyNAMiC is planned to be a longitudinal study with three measurement occasions separated by 5 years. It examines the relation of D1DRs, the functional connectome, and other brain parameters (e.g. gray- and white matter volume, white matter microstructure, and perfusion) to cognitive measures across the adult lifespan. 180 healthy participants covering the adult lifespan (age 20 - 78 years old) were assessed with an extensive cognitive test battery. Moreover, all participants underwent MRI for various neuroimaging markers along with D1DR assessment using [11C]SHC23390 and PET. A subset of elderly participants (n=20, age 65 - 77 years) received an additional assessment of dopamine D2/3DR receptor availability using [11C]raclopride. RESULTS: Based on previous post-mortem studies, but in contrast to a recent meta-analyses (Karrer et al), we predicted an average difference of approximately 5 - 10% per decade for D1DRs in prefrontal and striatal regions. We further expected that reductions to D1DRs would be regionally distinctive. An assumption-free general additive model analysis revealed non-linear

age-related trajectories for several cortical and subcortical regions with steeper D1DR decline in early adulthood (<40 years old) followed by slower rate of decline. Further shape analyses revealed remarkable similarity in D1DR trajectories across different regions at early adulthood followed by more divergent trajectories at older age. These findings may suggest that D1DRs differences in early adulthood reflect some degrees of post-developmental events, which is in good concordance with gene expression studies exhibiting protracted D1DR development. Despite striking similarity in D1DR trajectories in early adulthood across striatal regions, the non-linear age-related trajectory was less pronounced for the caudate compared to putamen, suggesting that the former might be more susceptible to early age-related insult. In support of this, we found that reduced cardiovascular health was associated with differences in D1DR in caudate, but not in putamen. CONCLUSIONS: Our finding may suggest a bi-phasic model of age-related D1DRS, and thus challenges the contemporary models supporting a single linear continuum of D1DRs differences across the adult lifespan.

Parallel Session 9 – Dopamine signal complexities in learning and reward: From model-free to model-based and somewhat in between

Dopamine release reflects value-less prediction errors during sensory-sensory learning

Kauê Costa[1], Geoffrey Schoenbaum[1]
[1]National Institute on Drug Abuse Intramural Research Program

BACKGROUND AND AIM: Most learning studies focus on value-based associations, where cues predict explicitly relevant outcomes, like rewards or punishments. Less is known about the mechanisms of learning in the absence of value. Dopamine release in the ventromedial striatum (VMS) is known to reflect value-based prediction errors, but it is not known if it also encodes errors in value-less information. METHODS: To address this open question, we used fiber photometry and dLight1.2 to record VMS dopamine release in rats during a sensory pre-conditioning task, which by definition relies on the learning of value-less sensory-sensory associations. Rats were first exposed to two pairs of neutral contiguous auditory cues, A-B and C-D (pre-conditioning), then underwent discriminative conditioning, where B was paired with food and D was not, and finally a probe test in which all cues were presented. RESULTS: Food-port responding during each cue was similar in pre-conditioning, and in conditioning rats progressively responded more to B than D. In the probe, most rats responded more during A than C, indicating they learned the A-B association and used this to infer that A might also lead to reward (as it predicted B). In the first pre-conditioning trials, we observed dopamine peaks at each cue onset, and, as learning progressed, these peaks diminished, with responses to predicted events (B and D) diminishing faster than those to unpredicted stimuli (A and C). At the start of conditioning, dopamine responses to B and D were partially reinstated, indicating that their previous reduction was also dependent on their predictability, and not only to general effects on habituation, salience, or novelty. The degree to which responses to B were diminished during preconditioning (a measure of prediction error) was also correlated with the relative responding to A during the probe, indicating that this apparent value-less prediction error signaling may drive sensory-sensory learning. During conditioning, dopamine signal dynamics in the same recording sites showed clear reward prediction error correlates, demonstrating that both dopamine responses during value-based and value-less learning were co-localized in the same general brain region. Importantly, the few rats that showed no evidence of learning the A-B association in the probe session also had less suppression of dopamine

responses to B in pre-conditioning and lower dopamine responses to A during the probe. CONCLUSIONS: Our results suggest that VMS dopamine release may reflect a general prediction error term that encompasses both value-based and value-less experiences. We are currently working on a computational model that would unify these different teaching signals into a single normative framework

Characterizing sensory prediction errors in the human midbrain and their role in stimulus-outcome learning

Thorsten Kahnt[1], James D. Howard[2], Javier A. Suarez[2], Geoffrey Schoenbaum[3]
[1]National Institute on Drug Abuse Intramural Research Program, [2]Northwestern University, [3]NIDA

BACKGROUND AND AIM: There is general consensus that midbrain dopamine neurons signal reward prediction errors. However, recent work in rodents has shown that these neurons also respond to errors in the prediction of value-neutral outcome features, such as identity. METHODS: In two experiments, we used Pavlovian transreinforcer reversal learning tasks involving rewarding food odors, computational modeling, and functional magnetic resonance imaging (fMRI) to test whether the human midbrain responds to violations in expected outcome identity. RESULTS: We found that midbrain fMRI responses correlate with errors in both value and identity predictions, suggesting a common neural origin of these signals. Moreover, across time and subjects, midbrain identity errors correlate with updates in identity expectations encoded in the orbitofrontal cortex (OFC). Importantly, although midbrain error signals do not scale with the perceptual distance between expected and received outcomes, they contain specific information about the identity of the mispredicted outcome. CONCLUSIONS: These findings demonstrate that the human midbrain responds to errors in value-neutral features of predicted outcomes, and support the idea that sensory prediction errors support the formation of stimulus-outcome associations in the OFC. Together, they suggest a broader role for midbrain dopamine in associative learning beyond the model-free learning of cached values.

Beyond model-free reinforcement learning: representation of task structure by rats working for rewarding optical stimulation of dopamine neurons

Peter Shizgal[1], Fancis Carter[1], Vasilis Pallikaras[1]
[1]Concordia University

BACKGROUND AND AIM: In model-free reinforcement learning, the value of the actions available in different world states is learned incrementally from experience by trial and error. Reward-prediction errors mediated by phasic dopamine (DA) signaling have been proposed as the basis of model-free learning. I will review experiments in which rats learn the relationship between different world states on the basis of optically induced activity of midbrain dopamine neurons. METHODS: The rats were trained to hold down a lever for 2 s to receive an optical reward: a 1 s train of 5 ms, 473 nm pulses delivered to midbrain dopamine neurons expressing channelrhodopsin-2. The 45 trials per session consisted of a 4 min interval during which a reward of constant strength could be earned by holding down a lever for 2 s. The trials were grouped into 15 repeating triads. The strength of the reward was predictable on leading and trailing trials of each triad but unpredictable on middle trials. RESULTS and CONCLUSION: In contrast to the behavior of an agent performing model-free learning, the rats showed evidence of having learned

the task structure as well as very rapid updating once the strength of the stimulation on the middle trials was revealed.

Model-based predictions for dopamine

Angela Langdon[1], Melissa Sharpe[2], Geoffrey Schoenbaum[3], Yael Niv[1]
[1]Princeton University, [2]University of California, Los Angeles, [3]NIDA

BACKGROUND AND AIM: Phasic dopamine responses are thought to encode a prediction-error signal consistent with model-free reinforcement learning theories. However, a number of recent findings highlight the influence of model-based computations on dopamine responses, presenting a challenge to our current understanding of reward prediction and learning in the brain. METHODS: I will discuss several features of phasic dopamine responses that suggest the influence of 'internal models' on reward prediction error correlates in the midbrain, and introduce model-based reinforcement learning theories that can make sense of these surprising features. RESULTS: Across a number of tasks, I will show how dopaminergic reward prediction errors reflect more dimensions of an expected outcome than scalar reward value. These dimensions include reward identity, temporal delay and variability. Each of these features can be accounted for by relaxing the fundamental assumptions of model-free reinforcement learning, and introducing a framework in which reward learning is shaped by expectations about the structure of the task environment--'model-based' reinforcement learning. CONCLUSIONS: Our findings suggest that dopamine prediction errors are shaped by internal models of the structure of the task environment and reflect more dimensions of an expected outcome than scalar reward value. Rather than a strict dichotomy between model-based and model-free learning, we suggest that these may better be viewed as a set of intertwined computations rather than two alternative systems. I will discuss the computational implications of model-based learning with dopamine prediction errors and highlight open questions for understanding dopamine circuits in the brain.

Parallel Session 10 – SSRI antidepressants potentiate effects of psychostimulants on forebrain circuits and behavioral markers for addiction liability

Fluoxetine and other SSRI antidepressants potentiate addiction-related gene regulation by psychostimulant medications

Heinz Steiner[1]
[1]RFUMS/Chicago Medical School

Psychostimulants such as methylphenidate (MP) and SSRI antidepressants such as fluoxetine (FLX) are widely used in the treatment of neuropsychiatric disorders or as cognitive enhancers. They are also combined, for example, to treat ADHD/depression comorbidity. Preclinical work indicates that individually these drugs can have detrimental effects on brain and behavior, especially when used during brain development. However, few studies have investigated possible interactions between these drugs. This is surprising as their combined neurochemical effects (enhanced dopamine and serotonin action) are similar to effects of drugs such as cocaine. This talk reviews recent studies on the molecular effects of i.p. MP+FLX combinations in the rat forebrain. Our results show that combining FLX and other SSRIs with MP potentiates MP-induced gene regulation in corticostriatal circuits, mimicking effects of cocaine. These changes are present across most of the striatum, including nucleus accumbens, but are

most robust in the lateral, sensorimotor striatum. These findings suggest the potential for an enhanced addiction liability and other behavioral consequences for such combination treatments.

Juvenile administration of concomitant methylphenidate and fluoxetine alters behavioral reactivity to reward- and mood-related stimuli and disrupts ventral tegmental area gene expression in adulthood

Carlos Bolanos[1]
[1]*Texas A&M University*

There is a rise in the concurrent use of methylphenidate (MP) and fluoxetine (FLX) in pediatric populations. However, the long-term neurobiological consequences of combined MP+FLX treatment during juvenile periods are unknown. Here, we present behavioral and molecular effects 1 and 60 days after MP+FLX treatment in preadolescent rats. MP+FLX exposure enhanced sensitivity to drug (i.e., cocaine, nicotine) and natural (i.e., sucrose) rewards, as well as anxiety- and stress-eliciting situations. MP+FLX enhanced the expression of extracellular signal-regulated protein kinase (ERK1/2) and its downstream targets CREB (cAMP response element binding protein), BDNF (brain-derived neurotrophic factor), and mTOR (mammalian target of rapamycin) within the ventral tegmental area (VTA) 1 and 60 days after drug treatment. Blockade of ERK2 activity rescued the MP+FLX-induced behavioral deficits. These results indicate that concomitant MP+FLX exposure during preadolescence increases sensitivity to reward-related stimuli while simultaneously enhancing susceptibility to stressful situations, and that these effects are due, at least in part, to long-lasting disruptions in ERK signaling within the VTA.

Chronic oral methylphenidate effects on functional brain connectivity, behavior and neurochemistry

Panayotis Thanos[1]
[1]*University at Buffalo*

Methylphenidate (MP) is extensively prescribed for attention deficit hyperactivity disorder (ADHD). However, illicit use of MP among healthy individuals has been increasing and is poorly understood in terms of its long-term effects on brain functional connectivity, neurochemistry and behavioral consequences. This talk reviews recent studies on awake brain glucose utilization, neurochemistry and behavior in healthy adolescent rats following chronic oral MP treatment. These studies utilized various methods such as PET imaging using [18F]fluorodeoxyglucose, as well as autoradiography and behavior testing. Our results show that chronic oral MP produces: a) increases in brain glucose utilization in the hippocampus, cerebellum and striatum, and resting state functional connectivity, b) behavioral, c) developmental changes as well as d) neurochemical changes (increased dopamine DAT and D1 receptor levels; increased microglial activation in distinct cortical and subcortical regions including globus pallidus, substantia nigra and caudate putamen. These findings support that MP and potentially in combination with SSRIs increase vulnerability for addiction and reward deficiency syndrome.

Methylphenidate and methylphenidate plus fluoxetine increase cocaine self-administration and trigger reinstatement of cocaine seeking behavior in rats

Micky Marinelli[1]
[1]*UT Austin*

Methylphenidate (MP) is commonly prescribed to treat attention deficit hyperactivity disorder (ADHD); it is also taken for non-medical purposes by those who use it recreationally or as a "cognitive enhancer". Combined exposure to MP and fluoxetine (FLX) is also common, for example, to treat ADHD/depression comorbidity or when persons taking FLX use MP for non-medical purposes. While the use of MP alone or in combination with FLX may be safe in some individuals, it is unclear if long-term exposure could subsequently increase the risk for cocaine addiction in some individuals, or if acute exposure could increase the risk of relapse in former cocaine users. In rat models, we show that repeated exposure to MP with or without FLX increases acquisition of cocaine self-administration, without changing motivation to self-administer cocaine. We also show that acute exposure to MP with or without FLX triggers relapse to cocaine seeking in rats previously trained to self-administer cocaine. In conclusion, MP with or without FLX can increase some aspects of cocaine self-administration and can trigger relapse to cocaine-seeking in subjects with a history of cocaine use.

Monday, May 23, 2022

Parallel Session 11 – Genetics and epigenetics of dopamine signaling and function

A coordinated dopamine-responsive gene expression program regulates neuronal function and cocaine response

Jeremy Day[1]
[1]University of Alabama at Birmingham

Drugs of abuse elevate dopamine levels in the nucleus accumbens and alter transcriptional programs believed to promote long-lasting synaptic and behavioral adaptations. However, the transcriptional response to dopamine receptor activation remains poorly understood, and the functional role of resulting gene expression programs has not been investigated due to technical limitations. Here, we employed bulk as well as single-nucleus RNA-seq in rat primary striatal neuron cultures to define a dopamine-regulated gene expression program. This program consists largely of CREB-responsive genes, is enriched for transcription factors, and occurs selectively in Drd1-positive medium spiny neurons. To dissect the function of this program, we engineered a large-scale multiplexed CRISPR activation strategy capable of coordinated and simultaneous upregulation of 16 dopamine-responsive genes. Induction of this gene program generated a secondary synapse-centric transcriptional wave that included altered regulation of ion channels, signaling proteins, and genes involved in synaptic plasticity. Using high-throughput in vitro multi-electrode arrays to record extracellular electrophysiological activity from thousands of neurons, we show that activation of this dopamine-dependent gene program alters selected physiological properties of striatal neurons, including an increase in the frequency of action potential burst events. Finally, using lentivirus-mediated delivery of CRISPR machinery into the nucleus accumbens, we show that induction of this gene program does not alter baseline locomotion or initial locomotor responses to cocaine, but enhances cocaine sensitization in vivo. These results provide proof of principle evidence that activity-dependent gene programs are sufficient to initiate both physiological and behavioral adaptations observed following experience with drugs of abuse.

Circadian rhythms in dopaminergic reward circuitry

Colleen McClung[1]
[1]University of Pittsburgh

Goals: The diurnal regulation of dopaminergic transmission is important for normal responses to salient stimuli. This diurnal rhythm can be disrupted in psychiatric disorders and addiction, contributing to disease pathophysiology. However, the molecular mechanisms that control these diurnal rhythms remain unclear. Methods: We used a combination of molecular, cellular, electrophysiological and behavioral approaches for this study. Results: We found that the CLOCK protein, a central regulator of circadian rhythms, directly regulates the transcription of tyrosine hydroxylase (TH) and dopaminergic activity in the ventral tegmental area (VTA). Moreover, this regulation of TH transcription by CLOCK is modified by the metabolic sensing, HDAC protein, Sirtuin 1 (SIRT1). This results in the regulation of diurnal rhythms in dopamine synthesis by the cellular redox state. Rhythms in TH and metabolic factors like NAD+ are highly disrupted following chronic cocaine administration, and CLOCK and SIRT1 together in the VTA are involved in modulating cocaine reward. We also found that the medium spiny neurons in the nucleus accumbens (NAc) display large diurnal differences in excitability and function, setting up a strongly diurnal dopaminergic reward circuit. Interestingly, these rhythms in the NAc do not depend on CLOCK but rather a different molecular mechanism, likely involving a related protein, NPAS2. Conclusions: These data demonstrate that the core circadian proteins are directly involved in regulating diurnal rhythms in dopaminergic function within the VTA-NAc reward circuit and they do so via interactions with epigenetic and metabolic factors. Disruption of these molecules or rhythms results in increased vulnerability for addiction-like behavior.

Dopaminylation of histone H3 in ventral tegmental area regulates cocaine-seeking

Ian Maze[1]
[1]Icahn School of Medicine at Mt. Sinai

Cocaine use disorder is characterized by enduring vulnerability to relapse during periods of attempted abstinence. This vulnerability is hypothesized to result from "rewiring" of brain reward circuitries, particularly ventral tegmental area (VTA) dopamine neurons, caused by long-lived changes in gene expression. Consistent with this hypothesis, histone mechanisms that control chromatin structures, and consequently gene expression patterns, have been shown to regulate various addiction-relevant behavioral abnormalities. Precisely how cocaine exposures act on midbrain dopamine neurons to precipitate addiction-relevant changes in gene expression is unclear. Given that histone H3 can be modified by monoamines on glutamine 5 (Q5) in response to fluctuations in their intracellular availability, we first sought to assess whether dopamine, like 5-HT, can be transferred to the unstructured H3 N-terminal tail. We find that this previously uncharacterized histone modification, H3 glutamine 5 dopaminylation (H3Q5dop), plays a critical role in cocaine-induced transcriptional plasticity in midbrain. Rats undergoing withdrawal from cocaine after a period of extended access to the drug showed an accumulation of intracellular dopamine concentrations and increased levels of H3Q5dop in VTA. A similar effect was observed in rats undergoing withdrawal from chronic heroin. By reducing H3Q5dop in VTA during withdrawal using viral mediated dominant negative approaches, we reversed cocaine-induced gene expression changes in VTA (as measured by RNA-seq), attenuated cue-induced dopamine release in nucleus accumbens (NAc)-as assessed by fast scanning cyclic voltammetry (FSCV)-

and reduced relapse-like cocaine-seeking behavior. These findings establish a neurotransmission-independent role for nuclear dopamine in regulating addiction-relevant transcriptional plasticity in VTA.

Activity-dependent epigenetic alterations underlying cocaine self-administration

Erin Calipari[1]
[1]Vanderbilt University

Substance use disorder (SUD) is a behavioral disorder characterized by aberrant learning about drugs and associated stimuli. At the core of this phenotype is the persistence of symptoms long after the cessation of drug use. The neural basis of these behaviors has been linked to receptor-based changes in neural circuits across the brain, however, the molecular drivers that allow for these changes to persist beyond the lifespan of any individual protein remain opaque. We recently identified a self-administration-induced remodeling of the transcriptome as well as a novel transcriptional signature that is induced by cocaine following a history of self-administration that predicts addictive behaviors. It will be critical to define the link between neuronal activation, and the longe-term changes in transcription that control drug seeking. Epigenetic adaptations - where DNA-protein interactions are modified to alter the probability of targeted transcription - have been implicated in the resilient nature of drug-seeking behavior. Histone acetylation, a generally permissive epigenetic mark, is induced following re-exposure to cocaine and cocaine-associated cues, suggesting that the epigenetic enzymes regulating histone acetylation are key regulators for drug-induced gene networks. Here we identify KAT2A, a histone acetyltransferase, as a potent regulator of the transcriptional networks that control drug-seeking. By combining transcriptional profiling with molecular approaches to identify KAT2A-induced histone modifications following drug self-administration, we identified changes in both KAT2A regulation of H3 and subsequent changes in H3 modifications that were predictive of seeking. Together, we have identified a critical regulator of activity-induced epigenetic reorganization by cocaine that controls addictive behaviors.

Parallel Session 12 – New ways of thinking about how to model addiction in laboratory animals

Behavioral markers of individual variation in motives for nicotine seeking in rats

Veronique Deroche-Gamonet[1]
[1]Université de Bordeaux

BACKGROUND AND AIM: Tobacco use leads to 6 million deaths every year due to severe long lasting diseases. Tobacco induces dependence, making smoking cessation difficult, even when 70% of smokers wish to do so. Even the most effective pharmacotherapies for smoking cessation have limited efficacy. Tobacco smoking is driven by different motives. It has been considered for long that these motives contribute equally in all smokers. However, converging data now support that individual variations may exist in their relative contribution. So far, individual variations have been poorly considered in animal models of nicotine seeking and taking, and, if so, it was exclusively through the study of quantitative variations (ie how much nicotine is seeked). We have questioned whether qualitative individual variations exist in the psychopharmacological mechanisms supporting nicotine intravenous self-administration in the rat. METHODS: Rats acquired intravenous nicotine self-administration behavior using nose-poke as the operant response, reinforced at FR3 by both nicotine (0.04 mg/kg/infusion) and

a salient light cue. After acquisition of stable behavior, the contribution of nicotine and the salient cue was tested through nicotine and cue omission tests. k-Means Cluster Analysis was run on variables of interest, and evidenced subpopulations were characterized and validated through a series of tests, which questioned the strength of nicotine primary reinforcing effects, cue secondary reinforcing effects and nicotine-induced reinforcement of the cue. RESULTS: In global populations of about 60 rats, we have consistently evidenced two subpopulations (about half of the population each), in which nicotine+cue self-administration was driven by different psychopharmacological mechanisms. For one subpopulation, self-administration was driven by nicotine-induced enhancement of the cue primary reinforcing effects, while in the second one, it was driven by the primary reinforcing effects of nicotine and the secondary reinforcing effects of the cue. CONCLUSIONS: We have evidenced individual variations in the psychopharmacological mechanisms supporting self-administration behavior in a preclinical model classically used to study nicotine seeking and taking. This work opens the perspective of exploring the neurobiological causal mechanisms for these individual variations, their long term impact on the development of nicotine dependence and whether approved treatments benefit more to one subpopulation than the other.

Reward-specificity of neuronal ensembles

Ana Clara Bobadilla[1]
[1]University of Wyoming

Background: Poorly regulated reward seeking is a central feature of substance use disorder. Convergent findings using different biomarkers reveal that only ~2-5% of cells encode a putative cocaine ensemble. When animals are exposed to two rewards, in vivo measurements of neuronal firing in the nucleus accumbens (NAcc) reveal ~20% overlap between neurons responding to self-administration of different types of reward such as cocaine, water, regular chow or sucrose, while a study using FISH reports that 50% of activated neurons in the infralimbic prefrontal cortex respond to both ethanol and saccharin. These results suggest a finely tuned specificity of ensembles. Here we comprehensively characterize the specific ensembles of neurons that are linked to reward seeking. We additionally address the question of whether addictive drugs usurp the neuronal networks recruited by natural rewards by evaluating cocaine- and sucrose-associated ensembles within the same animal. Methods: We use targeted recombination in active populations (TRAP) strategy, specifically FosCreERT2/+/Ai14 (cFos-TRAP) transgenic mice to deposit a cFos-driven Cre recombinase-tdTomato reporter into neurons activated during cue-induced reward seeking and extinction to tag cells as potentially encoding these behaviors. To characterize the seeking and extinction ensembles, these mice underwent the well-described rodent behavioral model of cocaine self-administration (SA), extinction training and cue-induced reinstatement of seeking. To define and compare different reward-specific ensembles within the same animal, we developed a poly-reward cocaine and sucrose self-administration paradigm in mice, where each reward is associated to a different discrete cue. After undergoing extinction training in absence of cues, mice are first re-exposed to one cue in presence of hydroxytamoxifen, allowing the tagging of the reward-specific ensemble with tdTomato, and a few days later exposed to the second cue, followed by immediate cFos tagging. Using this paradigm, we were able to assess the neurons included in the cocaine or sucrose ensembles, and to quantify the overlap between the two populations within the same animal exposed to both types of reward. Results: We tagged ~1% of neurons in the core subregion of the NAcc activated during cue-induced seeking for cocaine or sucrose. Most tagged cells in the

cocaine- or sucrose-seeking ensembles were D1-MSNs, and specifically activated during seeking, not when mice remained in the home cage. Using the cocaine and sucrose poly-reward model, we found ~70% distinction between the cells constituting the cocaine- compared to the sucrose-seeking ensemble, thus establishing ensembles are reward-specific. Conclusion: The data obtained here sheds new light on the ensembles in the NAcc sustaining maladaptive drug-oriented seeking behaviors and how it compares to natural rewards responses.

Amphetamine maintenance therapy for treating cocaine addiction: new insights into potential mechanisms

Anne-Noël Samaha[1], Florence Allain[1], Benoît Delignat-Lavaud[1], Marie-Pierre Beaudoin[1], Vincent Jacquemet[1], Terry Robinson[2], Louis-Eric Trudeau[1]
[1]Université de Montréal, [2]University of Michigan

BACKGROUND: D-amphetamine maintenance therapy shows promise as a treatment for people with cocaine addiction. Preclinical studies using Long Access (LgA) cocaine self-administration procedures suggest D-amphetamine may act by preventing tolerance to cocaine's effects at the dopamine transporter (DAT). However, Intermittent Access (IntA) cocaine self-administration better reflects human patterns of use, is especially effective in promoting addiction-relevant behaviors, and instead of tolerance, produces psychomotor, incentive, and neural sensitization. We asked, therefore, how D-amphetamine maintenance during IntA influences cocaine use and cocaine's potency at the DAT. METHODS: Male rats self-administered cocaine intermittently, with or without concomitant D-amphetamine maintenance therapy. This was followed by fast scan cyclic voltammetry recordings in the nucleus accumbens core to measure cocaine-induced inhibition of dopamine uptake at the DAT. RESULTS: Intermittent cocaine intake produced psychomotor sensitization, strong motivation to take and seek cocaine, and increased cocaine potency at the DAT. The co-administration of D-amphetamine suppressed both the psychomotor sensitization and high motivation for cocaine produced by IntA experience, and also reversed sensitization of cocaine's actions at the DAT, leaving baseline DAT function unchanged. CONCLUSIONS: Consistent with an incentive-sensitization view of addiction, our findings suggest that D-amphetamine might reduce cocaine use by preventing sensitization-related changes in cocaine potency at the DAT.

Central amygdala PKCδ-expressing neurons are critical to inhibition of incubation of methamphetamine craving after social choice-induced voluntary abstinence

Marco Venniro[1], Trinity Russell[1], Leslie Whitaker[1], Christopher Richie[1], Robert Messing[2], Yavin Shaham[1]
[1]NIDA, [2]University of Texas at Austin

Background: We recently reported that social choice-induced voluntary abstinence prevents incubation of methamphetamine craving. This protective effect was associated with activation of PKCδ- expressing neurons in central amygdala lateral (CeL) and inhibition of Fos expression in central amygdala medial (CeM). Here we used short-hairpin RNA against PKCδ mRNA (shPKCδ) and immunohistochemistry to determine the causal role of CeL PKCδ in inhibition of incubation of methamphetamine craving after voluntary abstinence. Methods: In Experiment 1 we used immunohistochemistry to validate the AAV virus expressing shPKCδ by injecting it (or shScram control) into CeL either 2 or 4 weeks before novel

context exposure to induce Fos. In Experiment 2, we trained two group of rats injected with shPKCδ or shScram into CeL to lever press for social interaction (6 d) and then for methamphetamine infusions (12 d). We then assessed relapse to methamphetamine seeking after 1 and 15 abstinence days. Between tests, the rats underwent social-choice-induced voluntary abstinence. After day 15 testing, we assessed Fos, PKCδ and Fos+PKCδ expression in CeL, and Fos expression in CeM. Results: In Exp. 1, we found that shPKCδ but not shScram decreased CeL PKCδ, Fos, and Fos+PKCδ expression. In Exp. 2, we found that shPKCδ but not shScram restored incubation of methamphetamine craving after voluntary abstinence. This effect was associated with decreased PKCδ, Fos and Fos+PKCδ expression in CeL, and increased Fos expression in CeM. Conclusion: Results demonstrate a critical role of CeL PKCδ in inhibition of incubation of methamphetamine craving after social choice-induced voluntary abstinence.

Parallel Session 13 – Forms and functions of glutamate and GABA co-release from midbrain dopamine neurons

Storage in different synaptic vesicles enables cotransmitters to encode distinct information

Kätlin Silm[1]
[1]Cedars-Sinai Medical Center

Corelease of multiple neurotransmitters has been described in many populations of neurons and often involves a fast, synaptically released neurotransmitter like glutamate or GABA along with a neuromodulator that is thought to act through volume transmission and signal changes in firing rate. The different modes of signaling have previously been ascribed to differences in the location and properties of receptors. It has not been known whether the two transmitters differ in the mode of release. Focusing on midbrain dopamine neurons, we showed that vesicles expressing transporters for monoamines (VMAT2) and glutamate (VGLUT2) differ in short-term plasticity, frequency dependence and coupling to presynaptic calcium channels. The ability to respond independently to the same neural activity results from the formation of two classes of synaptic vesicle through different endocytic pathways and this predicts that the two vesicle types contain different proteins. To identify the machinery responsible for the two release modes, we performed proteomics analysis of vesicles expressing VGLUT2 or VMAT2. The results begin to uncover the mechanisms responsible for differences in dopamine and glutamate release as well as release probability in general. They also reveal how, in the context of corelease, differences in release probability can be used to extract different information about the same neural activity and transmit the output as distinct signals.

Evidence for regulation of striatal dopamine release by co-released GABA

Jyoti Patel[1]
[1]NYU

Striatal dopamine (DA) axons co-release GABA (Tritsch et al. 2012). We tested the hypothesis that co-released GABA might autoregulate DA release via GABAA receptors on DA axons. To test this, we optically stimulated DA axons in dorsal and ventral striatum (dStr and vStr) in ex vivo slices from male and female Ai32:DAT-cre mice and monitored evoked increases in extracellular DA concentration ([DA]o) using fast-scan cyclic voltammetry. Application of the GABAA receptor agonist muscimol (10 µM) inhibited single-pulse (1 p) evoked [DA]o by 20-30% in both regions and in both sexes, suggesting the

presence of functional GABAA receptors on DA axons. Immuno-electron microscopy confirmed that DA axons in dStr and vStr express a3-GABAA- receptor subunits. The GABAA receptor Cl- channel blocker, picrotoxin (PTX; 100 µM), significantly increased 1 p evoked [DA]o in dStr and vStr in both sexes, consistent with a GABA tone in striatal slices. We then used optical pulse-train stimulation (10 p, 10 Hz) to assess the effect of co-released GABA on DA release throughout a stimulus train. PTX increased pulse-train evoked [DA]o throughout the striatum in both sexes, but to a greater extent than seen with 1 p. Moreover, the differential effect of PTX on 10 p versus 1 p was lost in mice lacking GAT1 in DA axons. These data provide evidence for autoregulation of DA release by co-released GABA in dStr and vStr, and introduce a3-GABAA receptors as mediators of this novel regulatory process.

Modulation of the dopamine neuron glutamate co-transmitting phenotype by aging and decreased dopamine synthesis.

Susana Mingote[1]
[1]City University of New York

Dopamine (DA) neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) use glutamate (GLU) as a co-transmitter. Early in development most DA neurons have the GLU co-transmitting phenotype, while in adulthood the density of DA-GLU neurons decreases to 30% in the VTA and 10% in the SNc. We are investigating factors that may alter the density of DA-GLU neurons in adulthood as a starting point to determine its effects at the circuit level. We first investigated the effects of aging. Using an intersectional viral strategy, we fluorescently labeled DA-only and DA-GLU neurons and found that the density of DA-GLU neurons decreased in the SN but not in the VTA in old aged mice. The number of transcripts of vesicular glutamate transporter 2 also decreased in the VTA, suggesting that aging affects GLU co-transmission in both regions. In parallel, we investigated how conditions associated with diseased states affect GLU co-transmission. Several disorders, including schizophrenia, show decreased DA synthesis in DA neurons projecting to the cortex. In mice, we found that the majority of cortical-projecting DA neurons have the capacity to co-release GLU. We wondered if decreasing DA synthesis would switch the DA-GLU phenotype into a GLU-only phenotype. To address this question, we produced a CRISPR/Cas9 deletion of tyrosine hydroxylase from cortical-projecting DA neurons and found that these neurons maintained their GLU phenotype. These studies reveal that DA neurons can dynamically regulate GLU co-transmission in adulthood and change their mode of communication with different brain regions.

Glutamate and dopamine co-release from mesoaccumbal glutamate projections differentially promote reward and aversion

Shelley Warlow[1]
[1]University of California San Diego

Ventral tegmental area (VTA) projections to the nucleus accumbens medial shell (NAc) drive reward-related motivation and positive reinforcement. Although primarily composed of dopamine neurons, a subset of mesoaccumbal dopamine projections also express the type 2 vesicular glutamate transporter (VGLUT2) and release glutamate at NAc terminals. Optogenetic stimulation of VGLUT2-expressing VTA neurons supports self-stimulation, but can also induce place avoidance, even in the same assay. Yet,

concomitant dopamine release by VGLUT2-expressing neurons is not required for self-stimulation, suggesting glutamate release is primarily responsible for promoting positive reinforcement. We first asked whether glutamate release by VTA glutamate neurons, subsets of which co-release dopamine or GABA, is necessary to support the optogenetic self-stimulation of VTA VGLUT2 neurons. We expressed Cre-dependent Channelrhodopsin (ChR2) in VTA of VGLUT2-Cre mice, in combination with CRISPR/Cas9 to disrupt the gene encoding VGLUT2. We found that disruption of VTA VGLUT2 effectively eliminated optogenetic triggered excitatory postsynaptic currents in NAc, while preserving co-released dopamine. Loss of VGLUT2 also abolished optogenetic self-stimulation, but left real-time place avoidance intact. Separately, CRISPR/Cas9 deletion of Tyrosine hydroxylase (Th) from VTA VGLUT2 neurons preserved optogenetic self-stimulation while abolishing place avoidance. Finally, CRISPR/Cas9 deletion of both Th and VGLUT2 abolished both self-stimulation and place avoidance. Our results demonstrate that glutamate release from VTA VGLUT2 neurons is positively reinforcing, but that dopamine release from these same neurons can induce avoidance behaviors.

Parallel Session 14 – DAT's so complex: Insights into dopaminergic pathophysiology and treatments from the study of dopamine transporter-targeted drugs, regulators and mutations

Sex and the Circuitry: Dopamine Transporter Regulation In Vivo

Randy Blakely[1]
[1]Florida Atlantic University Brain Institute

The presynaptic dopamine (DA) transporter (DAT) exerts significant regulatory control over DA signaling. Disrupted DAT regulation is suggested to contribute to multiple neurobehavioral disorders including ADHD, Autism, Bipolar Disorder and Schizophrenia. At the DA neuron cell soma and at DA synapses, D2-type DA receptors (D2Ars) regulate many aspects of DA neural signaling capacity, including DA synthesis, DA vesicular release, and DAT phosphorylation linked to DAT-mediated DA transport, efflux and clearance. Recently, we demonstrated that D2AR regulation of DAT phosphorylation and surface trafficking is readily observed in the dorsal striatum of male mice, but not in the ventral striatum, a specificity that impacts the penetrance of a human DAT coding variant (DAT Val559) as well as the actions of a D2AR directed medication. In more recent studies, age and sex-dependence in D2AR regulation has also become evident. Age, region and sex-specificities of D2AR-dependent DAT regulation appear also to drive differential neural plasticities arising from DAT dysfunction, measures of cognitive function, and behavioral responses to psychostimulants. I will discuss these and ongoing studies, with specific attention to how our prior assumptions about the homogeneity of DAT regulation must be refined to understand pathophysiology and precision medication development.

Novel adaptations in dopamine transporters and associated proteins following high-dose, extended-access cocaine self-administration

Sara Jones[1]
[1]Wake Forest University

Long-access cocaine self-administration in rats produces many of the hallmark brain changes found in human cocaine abusers, including changes in dopamine transporter (DAT) function and sensitivity to cocaine. We have found that extensive exposure to cocaine induces the formation of DAT homo-

oligomers, and these oligomers are sub-sensitive to cocaine's uptake inhibition properties. This results in tolerance, escalation of self-administration and transition to more compulsive drug seeking behaviors. These changes can be reversed by administration of releasers such as amphetamine or phenmetrazine, through the dissociation of DAT oligomers. Further, motivation to self-administer cocaine on a progressive ratio schedule is tightly correlated with DAT levels at the plasma membrane, leading us to evaluate the involvement of DAT-associated proteins in regulating dopamine uptake kinetics and pharmacology. Recent work has shown that the level of a DAT-binding integral synaptic vesicle protein, synaptogyrin-3 (SYG3), is strongly positively correlated with both DAT levels and motivation to take cocaine, suggesting that DAT-SYG3 interactions may be involved in uncontrolled drug-taking and seeking behaviors. We will explore DAT-SYG3 interactions and their relationship to multiple aspects of DA neurotransmission and drug self-administration.

Watch Flies Teaching Us Mechanisms of Neuropsychiatric Disorders

Aurelio Galli[1]
[1]*University of Alabama at Birmingham*

The human dopamine (DA) transporter (hDAT) mediates clearance of DA. Genetic variants in hDAT have been associated with DA dysfunction, a complication associated to several brain disorders including autism spectrum disorder (ASD). Here, we investigated the structural and behavioral bases of an ASD-associated in-frame deletion in hDAT at N336 (ΔN336). We uncovered that the deletion promoted a previously unobserved conformation of the intracellular gate of the transporter, likely representing the rate limiting step of the transport process. It is defined by a "half-open and inward facing" state (HOIF) of the intracellular gate that is stabilized by a network of interactions conserved phylogenetically, as we demonstrated it both in hDAT by Rosetta molecular modeling and fine grained simulations as well as in its bacterial homolog leucine transporter by EPR analysis and X-ray crystallography. The stabilization of the HOIF state is associated with both DA dysfunctions demonstrated in isolated brains of Drosophila melanogaster expressing hDAT ΔN336 and with abnormal behaviors observed at high-time resolution. These flies display increased fear, impaired social interactions, and locomotion traits we associate with DA dysfunction and the HOIF state. Together, our results describe how a genetic variation causes DA dysfunction and abnormal behaviors by stabilizing a HOIF state of the transporter.

Dopamine transporter deficiency syndrome; pharmacological chaperones and a new animal model.

Ali Salahpour[1]
[1]*University of Toronto*

Introduction: Hereditary dopamine transporter deficiency syndrome (DTDS) is a genetic condition caused by loss-of-function mutations in the dopamine transporter (DAT). The disorder is characterized by parkinsonism-dystonia and raised cerebrospinal fluid levels of dopamine metabolites. No treatment is currently available and patients generally do not survive past adolescence. When expressed in vitro, the DAT missense mutations result in reduction of dopamine uptake as well as preventing DAT protein maturation. In this study, we aimed to identify pharmacological chaperones of DAT as a potential treatment for DTDS. This approach has been used previously to rescue misfolding mutations causing cystic fibrosis and Nephrogenic diabetes insipidus. Methods/Results: We have recently shown that

ibogaine and bupropion can increase surface expression and activity of wild type and some DTDS disease causing mutants. Importantly, ibogaine and bupropion treatment increase mature protein and uptake of mutants suggesting that these compounds are pharmacological chaperones of DAT in heterologous cells. To complement these studies, we have recently generated a mouse model of DTDS by knocking in the A313V mutant which can be rescued by bupropion and ibogaine in cells. Our characterisation so far shows that A313V-KI DAT mutant mice have an 80% reduction in striatal and midbrain mature DAT protein levels compared to WT animals. Importantly, we also detect the presence of immature DAT protein in the midbrain samples of the A313V mutants while no immature band is observed in WT animals. Behaviourally, A313V-KI mice display mild hyperactivity and blunted response to amphetamine in agreement with their reduced DAT levels. Conclusion: Our results suggest that pharmacological chaperones are a viable approach for the treatment of DTDS. The new A313V-KI mouse will be an important tool to allow us to better understand the physiological consequences of DTDS mutations and identify pharmacological agents for the treatment of this disease.

Parallel Session 15 – Serotonin and dopamine interactions in Parkinson's disease

The role of glutamate co-transmission by serotonin neurons of the dorsal raphe nucleus in the expression of L-Dopa-induced dyskinesia

Martin Parent[1]
[1]*Université Laval*

BACKGROUND AND AIM: Parkinson's disease is characterized by the progressive loss of midbrain dopaminergic neurons that innervate the striatum. The dopamine precursor L-3,4-dihydroxyphenylalanine (L-Dopa) is the most effective pharmacotherapy but its chronic use is hampered by adverse effects such as abnormal involuntary movements (AIMs), also termed L-Dopa-induced dyskinesia (LID). Recent studies have shown the crucial role of serotonin (5-HT) neurons in LID expression. Through this presentation, we will specifically address the functional role of glutamate co-transmission by 5-HT neurons of the dorsal raphe nucleus (DRN) in the regulation of motor behavior and LID expression. METHODS: We used CRIPSR-Cas9 technology and viral injections to knock-out or overexpress the atypical vesicular glutamate transporter 3 (VGluT3), specifically in 5-HT neurons of the dorsal raphe nucleus (DRN) in adult mice. After extensive behavioral testing, these mice were injected with 6-OHDA in the medial forebrain bundle to selectively lesion DA axons, and then treated with L-Dopa to induce AIMs. RESULTS: RT-qPCR assay, RNAscope and immunohistochemistry confirm the depletion or overexpression of VGluT3 in AAV-infected 5-HT neurons of the DRN. High-resolution confocal analysis of target sites reveals a decreased number of axon varicosities emitted by VGluT3-depleted 5-HT neurons. Before dopamine lesion and L-Dopa administration, VGluT3-depleted mice show increased spontaneous motor activity and impulsivity, as well as anhedonia. Compared to controls, VGluT3-depleted mice show lower motor disabilities induced by 6-OHDA and exacerbated AIMs caused by L-Dopa administration. CONCLUSIONS: Glutamate that is co-released by 5-HT neurons of the DRN appears to be involved the regulation of spontaneous motor behaviors and impulsivity, as well as in the expression of LID and anhedonia.

The 5-HT2A receptor, a unique therapeutic target to alleviate both dopaminergic psychosis and L-DOPA-induced dyskinesia in Parkinson's disease

Philippe Huot[1]
[1]McGill University

BACKGROUND AND AIM: With disease progression and years of dopamine replacement therapy with L-3,4-dihydroxyphenylalanine (L-DOPA), patients with advanced Parkinson's disease (PD) experience motor complications such as dyskinesia, as well as non-motor complications such as psychosis. Antagonising the serotonin 2A (5-HT2A) receptor appears as a promising strategy to alleviate both dyskinesia and psychosis, yet recent data from our laboratory indicate that there might be a ceiling to the effects conferred by this approach. Here, we review the pre-clinical and clinical literature that reported anti-dyskinetic and anti-psychotic effects of 5-HT2A blockade and seek to find ways to go beyond the apparent ceiling mentioned above. METHODS: We conducted experiments in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmosets exhibiting dyskinesia and psychosis-like behaviours (PLBs). After administration of L-DOPA in combination with the highly-selective 5-HT2A antagonist pruvanserin, we measured the severity of dyskinesia and PLBs. We selected the doses of pruvanserin to achieve plasma levels that ranged from below to above those that were reported to be well tolerated and to lead to near complete brain receptor occupancy in human. RESULTS: Pruvanserin exhibited robust anti-dyskinetic and anti-psychotic properties. However, it was impossible to reduce dyskinesia by more than 60% and PLBs by more than 55%, even at doses 100-fold higher than those leading to clinically-relevant plasma levels. Furthermore, at such high doses, pruvanserin mildly interfered with L-DOPA anti-parkinsonian benefit. CONCLUSIONS: Whereas 5-HT2A blockade is a strategy that may alleviate both L-DOPA-induced dyskinesia and dopaminergic psychosis, our results indicate that it may not be possible to eradicate dyskinesia and psychosis in PD through a 5-HT2A-selective approach.

Targeting serotonin neuroplasticity to optimize L-DOPA treatment in Parkinson's disease

Christopher Bishop[1]
[1]Binghamton University

BACKGROUND & AIM: Parkinson's disease is typified by a constellation of motor symptoms that are usually well-treated by the dopamine (DA) replacement therapy L-DOPA. However, chronic L-DOPA treatment precipitates debilitating and often intractable side effects such as choreic and dystonic movements called L-DOPA-induced dyskinesia (LID). While the mechanisms underlying LID are multifaceted, reorganization of the serotonin (5-HT) system has been strongly implicated. In this talk I will present a brief overview of 5-HT neuroplasticity in Parkinson's disease and LID as well as provide emerging strategies for targeting aberrant 5-HT compensation for the improvement of L-DOPA treatment for Parkinson's disease patients. METHODS: We employed the rat hemi-parkinsonian model of LID to examine whether pharmacological and/or genetic 5-HT modulation could reduce the development or expression or LID while maintaining the benefits of L-DOPA. To do so, one cohort of rats was administered the FDA-approved anti-depressant Vilazodone, which acts as both a 5-HT1A receptor agonist and a 5-HT transport blocker. The second cohort received raphe-infused gene therapy to insert D2 auto-receptors in raphe-striatal terminals. Thereafter, behavioral, neurochemical and histological analyses were performed. RESULTS: We found that both prophylactic and interventional treatment with

Vilazodone significantly prevented and reversed established LID without altering L-DOPA efficacy. In rats receiving gene therapy, D2 receptor transfection led to pronounced attenuation of LID development and normalization of L-DOPA-induced striatal DA release but did not alter L-DOPA's anti-parkinsonian effects. CONCLUSIONS: Raphe-striatal 5-HT neuroplasticity is a risk factor for the expression of LID. By pharmacological or genetic targeting of this aberrant compensation we demonstrate an optimization of L-DOPA effects with clear translational potential for Parkinson's disease patients.

Differential impact of early or late lesions of the 5-HT system on parkinsonian symptoms
Véronique Sgambato[1]
[1]CNRS & Université de Lyon

BACKGROUND AND AIM: Serotonergic (5-HT) neurons degenerate in Parkinson's disease. Through this presentation, we will specifically address the role of this 5-HT impairment, besides the dopaminergic (DA) deficit, on the parkinsonian symptomatology. METHODS: We developed a macaque monkey model of Parkinson's disease exhibiting a double lesion (dopaminergic and serotonergic) thanks to the sequential use of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and MDMA (3,4-methylenedioxy-N-methamphetamine). We characterized this monkey model by multimodal imaging (PET, positron emission tomography with several radiotracers; DTI, diffusion tensor imaging), behavioral assessments (Parkinsonism, Dyskinesia, Neuropsychiatric-like behavior) and post-mortem analysis (with DA and 5-HT markers). RESULTS: When administrated after MPTP, MDMA injures the 5-HT presynaptic system without affecting the remaining DA neurons. The lesion of the 5-HT fibers induced by MDMA reduces both the motor (rigidity and dyskinesia) and the neuropsychiatric-like symptoms induced by levodopa therapy in MPTP-treated animals. Interestingly also, the administration of MDMA alone induces an anxious state, while its administration upstream of MPTP results in an aggravation of the parkinsonian deficits and associated DA injury. Dystonic postures, action tremor and global spontaneous activities were significantly affected. CONCLUSIONS: Late or early lesions of the 5-HT system do not have the same effects on parkinsonian symptoms in this animal model of Parkinson's disease.

Parallel Session 16 – Heterogeneity in dopamine neuron signaling

Mapping dopamine neuron synaptic connections in the striatum
Nao Chuhma[1], Stephen Rayport[1]
[1]Columbia University

Ventral midbrain dopamine (DA) neurons are a highly heterogeneous population using at least three small molecule cotransmitters: DA, glutamate and GABA. Recent genetic intersectional strategies have revealed that cotransmitting DA neuron subpopulations project to discrete striatal subregions differentially. While anatomical mapping of DA neuron projections is crucial for growing insights into neural circuit function, anatomical connectivity does not reveal functional connectivity nor the differential actions of the mix of transmitter and cotransmitters. To evaluate DA neuron functional connectivity systematically, we optogenetically activated DA neuron terminals in coronal slices of the mouse striatum, and recorded synaptic currents in the four major striatal cell types: direct-pathway and indirect-pathway spiny projection neurons, cholinergic interneurons, and fast-spiking GABA interneurons. Each transmitter receptor current was pharmacologically isolated, and recordings were

made from single identified striatal neurons distributed across the striatum, from the dorsal striatum to the nucleus accumbens and into the caudal tail of the striatum. We recorded a minimum of 100 neurons for each type of connection. DA neuron evoked GABA synaptic currents were observed broadly in the striatum in all four cell types, while DA synaptic currents were observed broadly but only in interneurons. Glutamate responses showed the greatest subregional heterogeneity, with hot spots in the medial nucleus accumbens and anterior-lateral-dorsal striatum. DA neuron synaptic connections to cholinergic interneurons were the strongest, and most complex, involving all three transmitters and their multiple receptors. This mapping reveals distinct striatal subregions with a different DA neuron synaptic language based on the mix of transmitters released, striatal cell types, and their receptors.

Distributional reinforcement learning through structured variability in dopamine signals

Naoshige Uchida[1]
[1]Harvard University

Recent studies in artificial intelligence have developed novel algorithms that that proved efficient empirically in silico. This raises the question whether these algorithms are used in the brain. Distributional reinforcement learning (RL), a novel RL algorithm, is shown to improve AI performance in bench mark tests based on video games. In environments in which rewards are probabilistic, traditional RL algorithms have focused on learning to predict a single quantity, the average over all potential rewards. Distributional RL, by contrast, learns to predict the entire distribution over rewards by employing multiple value predictors that together encode all possible levels of future reward concurrently. Remarkably, theoretical work has shown that a class of distributional RL, called 'quantile distributional RL', can arise out of a simple modification of traditional RL that introduces structured variability in dopamine reward prediction error (RPE) signals. In this talk, I will discuss our recent study that presents evidence that the brain's dopamine system uses distributional RL.

Dopamine diversity within the substantial nigra

Jochen Roeper[1]
[1]Goethe University Frankfurt

While diversity of dopamine (DA) neurons in the rodent VTA is by now well established, the DA neurons in the substantial nigra - apart from a small percentage of calbindin-immunopositive neurons are often assumed to be fairly homogeneous. Using a combination of in vivo and in vitro single cell recordings techniques, including recently established in vivo patch-clamp recording, we characterised the functional properties of identified DA SN neurons. First, we demonstrated that nigrostriatal DA neurons in the lateral SN displayed enhanced in vivo bursting in comparison to those in the medial SN, even when sharing the same major axonal projection to the dorsolateral striatum (Farassat et al. 2019 elife). In vivo pharmacology demonstrated that only lateral but not medial DA SN neurons are sensitive to low nanomolar concentration of the L-type channel blocker isradipine, In corresponding in vitro recordings, we identified Cav1.3 as the relevant calcium channel for boosting burst-like discharge. In addition, lateral SN DA neurons possess the highest rebound excitability mediated by T-type calcium channels. Indeed, we observed spontaneous rebound bursting occurring in DA SN neurons with in vivo patch-

clamp recording. In summary, lateral DA SN neurons show unique excitability among DA midbrain neurons, which might shed new light on their function and high vulnerability in Parkinson Disease.

Measuring the 3-dimensional topography of functional DA signaling during instrumental learning and behavior

Mark Howe[1]
[1]Boston University

Measurements and manipulations of striatum dopamine (DA) signaling have indicated that relationships between DA dynamics and behaviorally relevant variables such as movements, cues, and rewards differ across striatal subregions. Recent evidence has come from studies using genetically encoded sensors which enable optical measurements of spatially restricted DA signals in awake, behaving animals. However, conventional optical approaches such as fiber photometry have been generally limited to measuring signals from a small volume of striatum tissue around the tip of a single optical fiber. To better capture the complete spatiotemporal topography of striatal DA signaling and its relationship to behavior, we have developed and validated a new optical approach for measuring DA signals at many locations simultaneously across the full 3-dimensional volume of the striatum in awake, behaving mice. I will present preliminary results which provide new insight into the spatial variations in DA signaling during aspects of instrumental learning and performance.

Parallel Session 17 – Inhibitory modulation of dopamine neurons of the substantia nigra

Dendrite-specific inhibition of dopaminergic neurons

Rebekah Evans[1]
[1]Georgetown University

Retrograde viral tracing studies show that dopaminergic neurons receive inhibitory input from many nuclei in the basal ganglia. Using two-photon imaging, spatially-specific optogenetic stimulation, and electrophysiology to test the functional strength, receptors activated, and dendritic location of two basal ganglia inputs. We compare the strength and location of genetically-defined inhibitory subpopulations in the striatum (striosome and matrix) and globus pallidus (Parvalbumin and Lhx6). We find that the striosomal inputs selectively inhibit the ventrally-projecting "SNr dendrite" of the dopamine neurons. Although isolated to the SNr dendrite, this connection exerts strong control over the entire cell, pausing action potentials and facilitating rebound firing. By contrast, the Lhx6 neurons of the globus pallidus inhibit dopamine neurons at the soma and proximal dendrites but do not result in rebound firing. We find that striosomal input facilitates rebound firing because it activates GABAb receptors, which strongly hyperpolarize the SNr dendrite. Because the globus pallidus inputs selectively activate GABAa receptors, they pause firing without recruiting rebound conductances. We use neural reconstructions and computational modeling to show that the "SNr dendrites" of the dopaminergic neurons are specialized for receiving inhibitory signals, while the "SNc dendrites" are specialized for receiving excitatory signals. In addition, the computational model shows that dendrite-specific inhibition more effectively generates rebound action potentials than either somatic inhibition or broad inhibition of the entire dendritic arbor. Finally, we show that striosomes preferentially inhibit the subset of SNc

dopamine neurons which express intrinsic rebound mechanisms. Therefore, we describe a dendrite-specific striatonigral circuit for generating dopamine neuron rebound activity.

Cannabinoid Receptor 1 is required for neurodevelopment of the striatonigral dopamine system

Jill Crittenden[1]
[1]Massachussetts Institute of Technology

The midbrain substantia nigra pars reticulata (SNr) is composed of basal ganglia output neurons that control motor behaviors. Dopamine is released locally within the SNr from dendrites that descend ventrally from the overlying substantia nigra pars compacta (SNc). The function of dendritic dopamine release is unknown but presumably works by signaling through D2 receptors located on the dopamine neurons themselves and D1 receptors located on incoming axons from the striatum. Striatal inputs to the SNr arise from two striatal compartments, known as the striosomes (or patches) and matrix, that differ in many ways including their responses to dopamine stimulation, neural circuitry, and vulnerability to neurological diseases. Projections from neurons in the matrix comprise the classical direct pathway to SNr output neurons. By contrast, the striosomal axons climb bundles of dendrites in the SNr that descend from dopamine-containing SNc neurons, thereby forming the conspicuous striosome-dendron bouquets that are even visible in the Paxinos atlas. We previously showed that the CB1R cannabinoid receptor, which is known to inhibit presynaptic neurotransmitter release, is enriched in the GABAergic striosomal axons of adult mice. We now find that CB1R becomes enriched in striosomal projection neurons in the early postnatal period and that this corresponds to the time of striosome-dendron bouquet formation. Moreover, striosome-dendron bouquets are severely disorganized in knockout mice that lack CB1R expression, despite grossly normal striosomal cell numbers and organization of striosome and matrix compartments in the striatum. These data suggest that cannabinoid signaling plays a major role in early postnatal development of the striatonigral dopamine system.

Using a novel PV-Cre rat model to characterize pallidonigral cells and their terminations

Fumino Fujiyama[1]
[1]Doshisha University

In the present study, we generated a novel parvalbumin (PV)-Cre rat model and conducted a detailed morphological and electrophysiological investigation of axons from PV-globus pallidus (GP). The GP is considered a relay nucleus in the indirect pathway of the basal ganglia (BG). Previous studies have used molecular profiling and projection patterns to demonstrate cellular heterogeneity in the GP; for example, PV-expressing neurons are known to comprise approximately 50% of GP neurons and represent majority of prototypic neurons that project to the subthalamic nucleus and/or output nuclei of BG, entopeduncular nucleus and substantia nigra (SN). The present study aimed to identify the characteristic projection patterns of PV neurons in the GP (PV-GP neurons) and determine whether these neurons target dopaminergic or GABAergic neurons in SN pars compacta (SNc) or reticulata (SNr), respectively. We initially found that 1) 57% of PV neurons co-expressed Lim-homeobox 6, 2) the PV-GP terminals were preferentially distributed in the ventral part of dorsal tier of SNc, 3) PV-GP neurons formed basket-like appositions with the somata of tyrosine hydroxylase, PV, calretinin and cholecystokinin immunoreactive neurons in the SN, and 4) in vitro whole cell recording during

optogenetic photo-stimulation of PV-GP terminals in SNc demonstrated that PV-GP neurons strongly inhibited dopamine neurons via GABAA receptors. These results suggest that dopamine neurons receive direct focal inputs from PV-GP prototypic neurons. The identification of high-contrast inhibitory systems on dopamine neurons might represent a key step toward understanding the BG function.

GABAergic inhibition of midbrain dopamine neurons by the pedunculopontine nucleus: Implications for motor behavior

Nadine Gut[1]
[1]Rutgers University

Midbrain dopamine (DA) neurons are essential for the modulation of goal directed actions by contributing to the balance of the direct and indirect striatal pathways. To this end, DA neurons encode positive and negative reward prediction error as well as movement itself. DA activity is therefore critical for normal motor function expressed in the context of the initiation and execution of specific and purposive behaviors. Essential for understanding how DA-dependent motor behavior is shaped is the understanding of the excitatory and inhibitory modulation that the DA midbrain receives from a wide variety of sources. Using different anatomical tracing techniques, we discovered a prominent, novel inhibitory input to dopamine neurons originating in the mesencephalic locomotor region and confirmed, by means of whole-cell recordings, an inhibitory, GABA-A dependent effect of this innervation on dopamine neurons. Using VGat-cre mice transduced with ChR2 in the PPN and implanted with optic fibers for optogenetic stimulation in the SNc, we examined the functional role of this pathway testing these mice in a battery of behavioral paradigms. Activation of this pathway abolished exploratory locomotion in the open field, however preserved other motor behaviors such as walking on the Rotarod or climbing down a vertical pole. Purposive behavior in form of goal directed action sequences was interrupted and movement vigor decreased. I will discuss the behavioral observations in the context of action control through the adjustment of the intrinsic value of ongoing actions and speculate about the implications of this pathway in movement disorders.

Parallel Session 18 – Heterogeneous ventral pallidum neurons and their control of dopamine signaling

GABAergic and glutamatergic projections from the Ventral Pallidum: two parallel pathways with opponent roles in motivated behaviors

Tom Hnasko[1]
[1]University of California San Diego

BACKGROUND AND AIM: The ventral pallidum (VP) is a structure central to reward pathways and the control of motivated behaviors. The VP is predominantly GABAergic but also includes cells expressing the glutamatergic marker vesicular glutamate transporter (VGLUT2). Using cell-type-specific tracing we found that VP glutamate and GABA neurons share similar main projections to the ventral tegmental area (VTA) and the lateral habenula (LHb). And preliminary data show that both Nac D1- and D2-MSNs functionally connect onto both VP cell types. However, using optogenetic manipulation, we observed that activation of VP GABA and glutamate cell bodies elicit opposite appetitive behaviors, reinforcement and avoidance respectively. And activation of VP GABA neurons, but not glutamate neurons, increased

Fos expression in VTA dopamine and glutamate neurons. METHODS: To better understand how VP neurons differentially drive behavior, we recorded calcium transients in VP GABA and glutamate neurons in freely behaving animals during rewarding or aversive events. Also, to shine light on how VP neurons modulate VTA cell population activity, we measured VTA Dopamine, GABA, and glutamate neuron calcium transients in response to VP GABA or glutamate neuron optogenetic activation. RESULTS: These new data suggest a role for both VP cell types in encoding the salience of appetitive events, while their activation induced differential consequences on the dopamine system and VTA neuronal population activity. CONCLUSIONS: These findings highlight a potent role for bidirectional control of motivated behaviors by collaborative VP inhibitory and excitatory neurons, dysregulation of which could contribute to the emergence of deficits in reward functions associated with drug addiction and other neuropsychiatric disease.

Differential regulation of drug seeking by subpopulations of ventral pallidum neurons

Jasper Heinsbroek[1]
[1]CU Denver, Anschutz Medical Campus

BACKGROUND AND AIM: Neuronal activity in the ventral pallidum (VP) drives relapse. The VP contains different types of GABAergic (VP GABA) and glutamatergic (VP Glu) projection neurons but the role of these cells during drug seeking remains unclear. METHODS: We trained mice to self-administer cocaine or heroin and used chemogenetics to manipulate different types of VP neurons during progressive ratio, extinction, and reinstatement tests. A subset of mice were implanted with gradient index lenses above the VP for miniscope calcium imaging during drug self-administration. RESULTS: Cocaine self-administration reduced inhibitory synaptic inputs onto VP GABA neurons, and stimulating VP GABA (or a subpopulation of these cells that express enkephalin [VP Penk]) increased the motivation for cocaine and elicited cocaine seeking in extinguished mice. By contrast, stimulating VP Glu prevented cocaine seeking. Calcium imaging showed different activation of these neuronal populations during cocaine seeking. VPGlu neurons increased their activity after extinction training, whereas VP GABA neurons showed increased calcium activity during reinstatement. Ongoing studies are investigating the roles of different VP neurons and their projections during heroin self-administration and relapse. CONCLUSIONS: These results confirm that the VP is a critical regulator for drug seeking behaviors and show that distinct aspects of drug-motivated states are mediated by different populations of VP neurons.

Ventral pallidum transcriptome adaptations after cocaine self-administration

Mary Kay Lobo[1]
[1]University of Maryland School of Medicine

BACKGROUND AND AIM: The ventral pallidum (VP) is critical for drug intake and seeking behavior. Repeated exposure to cocaine alters VP neuronal firing and neurotransmission but there is limited information on the molecular adaptations occurring in VP neurons following cocaine intake. METHODS: To provide insight into this we performed RNA-seq on VP of mice that underwent cocaine self-administration followed by twenty-four hours of abstinence. We then used RNAscope and RiboTag to examine gene expression in VP projection neuron subtypes after cocaine self-administration. Finally, we used adeno-associated viruses to target VP projection neurons and overexpress or CRISPR knockdown

an identified upregulated transcription factor, Nr4a1. RESULTS: We observed differential gene expression in 363 genes between animals that self-administered cocaine vs saline. Gene Ontology analysis uncovered alterations in synaptic and structural plasticity related genes. We then identified a common upstream regulator of these sets of genes, the transcription factor Nr4a1. Nr4a1 was increased in VP, specifically in VP neurons that project to mediodorsal thalamus (MDT), after cocaine self-administration. Consistent with this data, overexpression of Nr4a1 in the VP-MDT neurons enhanced drug seeking behavior after cocaine self-administration, while Nr4a1 CRSPR knockdown in these neurons reduced cocaine intake and drug seeking behavior. CONCLUSIONS: Altogether, our work provides new information into the molecular adaptations occurring in VP neurons in cocaine self-administration and relapse-like behavior.

Ventral Pallidal Circuits in Addiction-Relevant Appetitive and Aversive Motivation

Stephen Mahler[1]
[1]University of California, Irvine

BACKGROUND AND AIM: Drug addiction is characterized by excessive drug use despite harmful consequences, as well as inability to maintain abstinence after one makes a decision to quit. These symptoms rely upon an abnormal choice of drugs over alternative rewards, and over safety. Recent studies from our panelists and others show that VP contains neurons that mediate both reward seeking and aversive motivation--in part via their interactions with dopamine circuits. Specifically, VP GABA neurons and their projections to VTA seem to mediate approach and reward-motivated responding, without much apparent role in aversively motivated behaviors. METHODS: Here, we examine how VP GABA neurons in GAD1:Cre rats, and their projections to ventral tegmental area (VTA) and lateral habenula (LHb), participate in addiction-relevant responses to rewards, punishers, and cues for either. We chemogenetically inhibited or excited VP GABA neurons and examined effects on a range of cocaine, opioid (heroin or remifentanil), and food seeking behaviors. We also asked whether these neurons mediate responses to aversive shocks, or to localized or non-localized shock-associated stimuli (localized shock prod, or tone/shock fear conditioning). Finally, we determined how inhibiting them impacts mixed appetitive/aversive motivational states experienced during risky decision making, and reinstatement of cocaine or opioid seeking after shock-induced or extinction-based abstinence. RESULTS: Results indicate that VP GABA neurons play essential roles in maladaptive, addiction-related behaviors, especially when driven by conditioned stimuli. Inhibiting VP GABA neurons suppresses seeking of cocaine, opioids, and highly palatable foods, without effects on less-valuable foods, or low-effort consumption of heroin or remifentanil. Inhibiting these neurons does not affect perception or aversiveness of an electric shock, but surprisingly increases certain conditioned fear-like responses to shock-associated stimuli. CONCLUSIONS: Initial results suggest that VP GABA neuron projections to VTA, but not LHb are responsible specifically for appetitive motivation.

Parallel Session 19 – The development and disease of specific subtypes of dopamine neurons

Molecular mechanisms underlying subtype-specific dopamine neuron migration and substantia nigra development.

Jeroen Pasterkamp[1]
[1]University Medical Center Utrecht

Brignani S, Raj DDA, Schmidt ERE, Dudukcu O, Adolfs A, Ruiter AA, Rybiczka-Tesulov M, Verhagen M, Van der Meer C, Broekhoven M, Grossouw L, Pasterkamp RJ Department of Translational Neuroscience, UMC Utrecht Brain Center, Utrecht, The Netherlands BACKGROUND AND AIM: The midbrain dopamine (mDA) system is composed of molecularly and functionally distinct neuron subtypes that mediate specific behaviours and that show select disease vulnerability, including in schizophrenia and Parkinson's disease. Despite progress in identifying mDA neuron subtypes, how these neuronal subsets develop, wire up and organize into functional brain structures remains poorly understood. METHODS: Here we utilize a novel intersectional mouse genetic platform, Pitx3-ITC, in combination with fluorescent lightsheet microscopy and various other genetic mouse models to dissect the mechanisms of mDA neuron migration and substantia nigra (SN) development. RESULTS: Our data provide a novel genetic tool that allows select visualization of subtypes of SN mDA neurons. Further, they provide a unique 3D view of SN mDA migration during development and implicate the guidance molecule Netrin-1 in the migration and positioning of mDA neuron subtypes that populate the SN. Unexpectedly, we show that Netrin-1, produced in the forebrain and provided to the midbrain through axon projections, instructs the migration of GABAergic neurons into the ventral SN. This migration is required to restrict mDA neurons to their location in the dorsal SN. CONCLUSION: These data provide insight into the cellular and molecular basis of SN development and demonstrate that neuron migration can be controlled by remotely-produced and axon-derived secreted guidance cues, a principle that is likely to apply more generally.

The C2H2 type zinc finger transcription factor BCL11A defines a subset of midbrain dopaminergic neurons

Sandra Blaess[1]
[1]University of Bonn

Tolve M1, Ulusoy A2, Dernst A1, Bodea GO1, Liu P3, Khaled W4, Copeland N5, Baader S6, Di Monte D2, Blaess S1 1Institute of Reconstructive Neurobiology, Medical Faculty, University of Bonn, 2German Center for Neurodegenerative Diseases (DZNE), Bonn, 3School of Biomedical Sciences, The University of Hong Kong, 4Department of Pharmacology, Cambridge University 5MD Anderson Cancer Center, University of Texas, 6Institute of Anatomy, Medical Faculty, University of Bonn BACKGROUND AND AIM: Midbrain dopaminergic (mDA) neurons of the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) project to multiple target regions in the forebrain and modulate various behaviors. Transcription factor codes established during development may define the identity of specific mDA neurons subsets and may ultimately contribute to the determination of their projection targets, their functional properties and their susceptibility to neurodegeneration. We uncovered that Bcl11a, a C2H2 type zinc finger transcription factor, is expressed in a subset of mDA neurons in the lateral VTA and in the SNc in the developing and adult brain. METHODS: We are using a combination of intersectional labeling and tracing approaches, conditional gene inactivation and an alpha-synuclein overexpression model to determine whether Bcl11a-expressing mDA neurons form a distinct functional subset in the dopaminergic system. RESULTS: We find that both SNc- and VTA-Bcl11a-expressing mDA neurons contribute to specific subcircuits of the dopaminergic system. Inactivation of Bcl11a in mDA neurons

(Bcl11a cko mice) does not result in obvious changes in differentiation and maintenance of Bcl11a-mDA neurons, but Bcl11a cko mice show behavioral impairments suggesting that the loss of Bcl11a leads to functional deficits in mDA neurons. Moreover, Bcl11a could play a neuroprotective role within SN-mDA neurons, since overexpression of alpha-synuclein in the SNc leads to a significantly greater reduction in the number of SNc-mDA neurons in Bcl11a cko animals as compared to controls. CONCLUSION: We demonstrate that Bcl11a expression defines a specific subset of mDA neurons and show that the inactivation of Bcl11a in mDA neurons leads to behavioral changes and increased vulnerability to neurodegeneration in an alpha-synuclein overexpression model.

Axonal translatome in dopaminergic neuron sub-circuits reveal new mechanisms regulating dopaminergic axons pathfinding

Martin Lévesque[1]
[1]Université Laval

Gora C1, Patikas N2, Metzakopian E2, and Lévesque M1 1CERVO Brain Research Centre, Université Laval, Québec, Canada. 2UK Dementia Research Institute, University of Cambridge, UK. BACKGROUND: Recent single cell sequencing studies have identified diverse subpopulations among midbrain dopamine neurons. These dopamine neuron subsets establish distinct circuits in the brain but the mechanism controlling the precise organisation of these sub-circuits still remain unknown. During embryonic development, dopamine axons express specific guidance receptors that control their precise navigation in the brain. AIM: the goal of this study is to uncover the complete axonal translatome in dopamine neurons and to reveal new mechanisms controlling axon guidance in the dopamine system. METHODS: Using the ribotrap mice, in which ribosomes contained in dopamine neurons are tagged with a small peptide, we selectively isolated mRNA being translated in dopamine axons innervating different brain regions. RESULTS: Sequencing of these mRNA revealed the complete axonal translatome specific to the different dopaminergic sub-circuits. Our preliminary data indicate that a large part of the guidance machinery is locally translated in growing axons. CONCLUSIONS: At the light of our data, we propose new models to explain the precise organization of the nigrostriatal, mesolimbic and mesocortical dopaminergic pathways.

Modeling Parkinson's disease in midbrain-like organoids

Sarah Nickels[1]
[1]Université du Luxembourg

Schwamborn JC1 1RU Life Sciences, Université Luxembourg, Belvaux, Luxembourg BACKGROUND: In Parkinson's disease (PD) patients the dopaminergic neurons of one region in the midbrain, the substantia nigra, are highly vulnerable for degeneration, while the dopaminergic neurons of a neighboring region, the ventral tegmental area, are not. The reasons for this selective vulnerability are largely unknown to date. This lack of knowledge, to a good extend is the consequence of the absence of human specific models for the midbrain. AIM: We aimed at developing human midbrain organoids that recapitulate physiological and pathological processes of the human midbrain. METHODS: Here, we demonstrate that three-dimensional (3D) differentiation of expandable human midbrain floor plate neural progenitor cells (mfNPCs) leads to organoids that resemble key features of the human midbrain.

RESULTS: These organoids are composed of midbrain dopaminergic neurons (mDANs), which produce and secrete dopamine. Additionally, the midbrain organoids contain other neuronal subtypes, astrocytes and oligodendrocytes. They can be further enriched with induced pluripotent stem cell (iPSC) derived microglia. Patient and disease specific midbrain organoids can be generated through the usage of patient derived iPSCs. Importantly, in these disease specific organoids, key hallmarks of PD including reduced numbers of dopaminergic neurons and appearance of alpha-Synuclein positive protein aggregates are recapitulated. CONCLUSIONS: We provide a robust method to reproducibly generate 3D human midbrain organoids containing mDANs to investigate PD-relevant patho-mechanisms.

Parallel Session 20 – Dopamine as a mechanism linking early life adversity to psychopathology

Interactions between dopamine-related gene networks and early adversity influence endophenotypes and risk for adult disease

Patricia Silveira[1]
[1]McGill University

Dopaminergic neurons constitute a system underlying the brain response to both adverse and protective environmental conditions. To be able to investigate the role of dopamine on the long-term effects of early life adversity, we created a novel approach to genomic profiling, informed by biological function, and characterizing gene networks based on the levels of co-expression with a determined gene in a specific tissue. Lists of genes co-expressed with the gene of interest (components of dopamine neurotransmission such as DAT or its modulators like the insulin receptor) were created, and SNPs from these genes were compiled in polygenic scores using the association betas described in a GWAS. Across multiple samples, the biologically informed polygenic scores were calculated, and their ability to predict endophenotypes associated with metabolic and neuropsychiatric outcomes was investigated, according to variations in the levels of exposure to early life adversity. Our novel, biologically-informed approach integrates information from molecular neurobiology and GWAS technology, and enables the use of genomic datasets to probe relevant biological processes involved in neural function and disorders in response to adversity. Applying this novel approach to Developmental Neuropsychology and developmental origins of disease agenda guides the elaboration of more efficacious and cost-effective personalized prevention and treatment of complex disorders, targeting individuals that would benefit the most from interventions.

Drugs of abuse alter psychiatric risk in adolescence by disrupting dopamine development

Cecilia Flores[1]
[1]McGill University

Adolescence is a period of heightened vulnerability to develop psychopathology. This can be attributed in part to the fact that the brain, and most notably the medial prefrontal cortex dopamine circuitry, is still undergoing critical changes during this time. Several psychiatric conditions are disorders characterized by prefrontal cortex dysfunction. Nonetheless, there is a large gap in knowledge about the cellular and molecular processes mediating the maturation of prefrontal cortex dopamine circuitry during adolescence and how they are influenced by adverse experiences, including drugs of abuse and stress. In this talk I focus on the adolescent maturation of mesocorticolimbic dopamine neurons and on

the emerging role of the Netrin-1 guidance cue system and its microRNA regulators in the gradual unfolding of dopamine connectivity during this age, including dopamine axon tagging and pathfinding. I discuss how stimulant drugs of abuse alter these developmental events, inducing susceptibility or resilience later on in life, depending on the dose (recreational versus therapeutic-like doses), sex, and specific age within adolescence. Understanding when and how stimulant drugs interact with the developing male and female adolescent brain is particularly timely, since there is an increasing prevalence of stimulant use disorder and the pattern of transition from recreational to compulsive drug use appears to be sex and age dependent.

Astrocytic Regulation of Basal Ganglia Dopamine/D2-Dependent Behaviors

Zisis Bimpisidis[1]
[1]Istituto Italiano di Tecnologia

Astrocytic involvement in dopamine dynamics related to motivational and sensorimotor gating abilities is unknown. We found that dysbindin-1 (Dys1) hypofunction increases the activity of astrocytes, which express only the isoform Dys1A that is reduced in the caudate of patients with schizophrenia. Astrocytic Dys1A disruption resulted in avolition and sensorimotor gating deficits, increased astrocytic dopamine D2 receptors and decreased dopaminergic tone within basal ganglia. Notably, astrocytic Dys1A hypofunction disrupted dopamine dynamics linked to reward expectancy and interconnected with astrocytes Ca2+ responses mainly in the globus pallidus externus (GPe). Finally, we proved these phenotypes were mediated by D2 receptors in astrocytes as their selective deletion in astrocytes either in GPe or SNc/VTA enhanced motivation and sensorimotor gating abilities as well as dopaminergic release in the GPe. Therefore, astrocytes control motivational and sensorimotor gating processes through Dys1A/D2-dependent mechanisms within the basal ganglia.

Early life stress primes response to adult stress through ventral tegmental area epigenetic modifications

Catherine Peña[1]
[1]Princeton University

Early life stress (ELS) increases lifetime risk of depression and other mood, anxiety, and drug disorders by 2-4 -fold. We broadly hypothesize that ELS increases these risks through altered brain development, particularly within reward circuitry central to both depression and substance abuse disorders. Importantly, depression and other psychiatric syndromes emerge later in life. Studies suggest that ELS sensitizes individuals to subsequent stressful experiences. To study the molecular correlates of ELS-induced sensitivity within reward circuitry, we use a "two-hit" stress paradigm in mice. ELS-exposed mice display largely normal behavior at baseline, and increased depression-like behavior after additional stress in adulthood. This latent behavioral vulnerability is accompanied by latent transcriptional alterations in the ventral tegmental area (VTA). We hypothesized that such latent transcriptional alterations would be "primed" by chromatin modifications. Here, I will present unpublished data in which we profiled all possible histone modifications simultaneously using bottom-up mass spectrometry. The proportions of 14 histone modifications were altered by ELS, a majority of which are associated with open chromatin, in which genes are more readily transcribed. Among these, ELS

increased H3K4me3 and H3K4me1, marks of active and primed genomic regulatory elements. ChIP-seq for H3K4me1 revealed 209 differentially enriched peaks (FDR<0.05 and >20% fold-change), a majority of which were increased by ELS. Interestingly, there is greater correspondence between H3K4me1 enrichment and RNA-seq expression of putative enhancer-contacted genes after additional adult stress than after ELS alone, in support of a priming hypothesis. This research suggests novel epigenetic mechanisms mediating how ELS renders the VTA more reactive to future stress experience.

Tuesday, May 24, 2022

Parallel Session 21 – Recent insights into the importance of functional and anatomical heterogeneity of the dopamine system in behavioral control

Striatal Acetylcholine reports distinct update signals during flexible multi-step decision making

Lauren Burgeno[1]
[1]Netherlands Institute for Neuroscience

The striatum plays a critical role in coordinating reward guided decision making. It also has one of the highest concentration of markers for cholinergic transmission. Striatal acetylcholine (ACh), which is mainly supplied by a small population of cholinergic interneurons with extensive local arborization, exerts a powerful influence over neurotransmission and plasticity. A handful of electrophysiological studies show rewards and reward-predictive cues elicit ACh responses in simple behavioral tasks, and temporally coarse manipulations of striatal ACh suggest a role In rapid behavioral flexibility. However, historical technical challenges in measuring and precisely manipulating acetylcholine release in vivo have hampered the ability to refine our understanding of how striatal ACh shapes more complex behavior, such as when animals need to update sequential decisions in a structured environment. The recent advent of genetically encoded tools enabling measurement and manipulation of ACh levels with high temporal precision has rekindled interest in this area. Here we used the recently developed GRABACh3.0 sensor to characterize rapid ACh fluctuations in the nucleus accumbens core (NAc) and dorsomedial striatum (DMS) during a sequential reward guided decision-making task in mice. Probabilistic reward delivery enabled us to determine how ACh levels were shaped by reward expectations, and the action-state transition structure allowed us to measure ACh fluctuations while navigating changing action plans. Both NAc and DMS ACh carried time-locked information about (i) reward and reward expectations (though only by reward omission in DMS), (ii) value updates (inverse "reward prediction error"), (iii) action updates, and (iv) movement (at distinct timepoints in DMS and NAc). These signals co-occurred with sustained information reflecting the recent local reward rate. Together, these findings suggest that NAc and DMS striatal ACh differentially contribute to flexible decision making by signaling unexpected changes in the environment.

Nucleus accumbens acetylcholine modulates cue-evoked dopamine to regulate cue-motivated reward-seeking

Val Collins[1]
[1]University of California San Francisco

Heterogeneity of the midbrain dopamine system occurs at many levels. Recent work has highlighted a discrepancy between dopamine cell body activity and release, suggesting terminal modulation mechanisms are at play, refining the signal. The nucleus accumbens (NAc) cholinergic system is well suited to do so, as acetylcholine receptors are located directly on dopamine terminals and has been previously demonstrated to terminally modulate dopamine release. However, it remained unclear how this mechanism occurs in response to behaviorally relevant stimuli, and furthermore, what functional significance this mechanism had on behavior. Our work demonstrates how NAc acetylcholine receptor activity modulates behaviorally evoked dopamine release to regulate cue-motivated reward seeking, capable of acting as a restrictive gate. Collectively, these results elucidate the role of the NAc cholinergic system in regulating cue-evoked dopamine release to mediate the excitatory influence of a reward-paired cue to invigorate reward seeking and highlights the role of terminal modulation in contributing to the heterogeneity within the midbrain dopamine system.

Specialized dopamine projection neurons work cooperatively to maximize reward reinforcement

Larry Zweifel[1]
[1]University of Washington

Background and Aim: Dopamine neurons of the VTA regulate reward association and motivation. We aimed to establish whether these functions are regulated by distinct dopamine populations. Methods: Using mouse Cre-driver lines, we isolated dopamine subpopulations in the VTA and established connectivity in the ventral striatum. Optogenetics were used to establish the activity of two populations of neurons during instrumental conditioning and to determine the function of these neurons in behavioral reinforcement. Results: VTA-core projecting neurons facilitate Pavlovian reward learning and acquisition of an instrumental response. VTA-shell projecting neurons do not regulate Pavlovian reward learning and cannot facilitate acquisition of an instrumental response, but their activation can drive robust responding in a previously learned instrumental task. Both populations are activated simultaneously by cues, actions, and rewards and this co-activation is required for robust reinforcement of behavior. Conclusion: There are functionally distinct dopamine populations in the VTA for promoting motivation and reward association that operate on the same time scale to optimize behavioral reinforcement.

Dopamine reports reward prediction errors, but does not update policy, during inference-guided choice

Marta Blanco-Pozo[1]
[1]University of Oxford

Dopamine is thought to carry reward prediction errors (RPEs), which update values and hence modify future behaviour. However, updating values is not always the most efficient way of adapting to change. If previously encountered situations will be revisited in future, inferring that the state of the world has changed allows prior experience to be reused when situations are reencountered. To probe dopamine's involvement in such inference-based behavioural flexibility, we measured and manipulated dopamine while mice solved a sequential decision task using state inference. Dopamine was strongly influenced by the value of states and actions, consistent with RPE signalling, using value information that respected

task structure. However, though dopamine responded strongly to rewards, stimulating or inhibiting dopamine at the time of trial outcome had no effect on subsequent choice. Therefore, when inference guides choice, rewards have a dopamine-independent influence on policy through the information they carry about the world's state.

Parallel Session 22 – Dissecting the molecular regulation of dopamine release using innovative approaches to dopamine detection

Illuminating dopamine release at the synaptic level using a nanosensor paint

Sofia Elizarova[1], Nils Brose[1], Sebastian Kruss[2], James Daniel[1]
[1]Max Planck Institute of Experimental Medicine, [2]Georg-August University of Göttingen

BACKGROUND AND AIM: Despite substantial efforts to understand the molecular regulation of dopamine (DA) secretion in the brain, the molecular machinery of DA release from varicosities, and the molecular basis of the heterogeneity of varicosities, remain enigmatic. The study of DA release has been limited by the limited spatial resolution of classical methods of DA detection. To examine DA release at the level of single varicosities, we use ''Adsorbed Nanosensors Detecting Release of Dopamine' - AndromeDA. METHODS: This method uses immobilized nanosensors applied to dopaminergic neuronal cultures, which are electrically stimulated. AndromeDA arrays increase in fluorescence upon DA binding. AndromeDA nanosensor arrays are fluorescent in the near infrared, detect DA in the sub-nanomolar range and selectively report DA over other catecholamines. RESULTS: The 'painted' layer of nanosensors around cells allows the identification of individual DA release sites and a comparison of DA released from these sites. We detect both spontaneous and electrically evoked DA release, which can be resolved at single axonal varicosities. Detected DA release exhibits dependence on the stimulus strength, with high frequency stimulation or elevated extracellular K+ resulting in greater DA release than low frequency stimulation. Detected DA release is strongly decreased by reserpine treatment and enhanced by L-DOPA treatment. DA release varies between varicosities and many varicosities also do not exhibit detectable DA release, which is consistent with the presence of 'silent' varicosities. CONCLUSIONS: The use of AndromeDA arrays allow the examination of individual DA release sites with a high degree of spatial and temporal resolution. This paves the way for analyzing how DA release and the functional heterogeneity of dopaminergic varicosities is regulated by presynaptic proteins.

Resolving the time-course of dopamine release and receptor occupation with flash photolysis

Joe Lebowitz[1], Alex Condon[1], Brooks Robinson[1], Naeem Asad[2], Timothy Dore[2], Lin Tian[3], Kim Neve[1], John Williams[1]
[1]Oregon Health & Science University, [2]New York University Abu Dhabi, [3]UC Davis

Dopamine signaling in the somatodendritic compartment is mediated by D2-autoreceptors on the timescale of seconds, but concentration requirements suggest rapid and synchronous dopamine release underlies D2-IPSCs. Here, we describe the use of a caged inverse agonist (CyHQ-sulpiride) and flash photolysis to determine the time-course of synaptic dopamine release necessary to generate D2-IPSCs. Photoactivation of CyHQ-sulpiride terminated the current induced by dopamine superfusion with a decay constant similar to that of IPSCs. This suggests a short interaction time between dopamine and D2 autoreceptors, and this was tested by varying the time of photolysis relative to electrical stimulation of a

D2-IPSC. We found that while the duration of D2-IPSCs is ~1000 milliseconds, only flash photolysis of CyHQ-sulpiride within 90 ms of electrical stimulation reduced IPSC amplitude. 2-photon linescans of the fluorescence increase observed with the genetically encoded sensor dLight1.3b revealed a peak in 10 ms following a single stimulus and 45 ms following a pair of stimuli at 40Hz at hot-spots that were strikingly similar to striatal hot-spots. More recent and ongoing studies are utilizing CyHQ-sulpiride with newly discovered mutant D2-autoreceptors and agonists of varying affinities to distinguish ligand-receptor interactions from intracellular GPCR signaling cascades.

Complementary Roles of Synaptotagmins 1 and 7 in Somatodendritic Dopamine Release

Takuya Hikima[1], Paul Witkovsky[1], Margaret Rice[1]
[1]NYU Grossman School of Medicine

Somatodendritic release of DA in the substantia nigra pars compacta (SNc) acts on D2 autoreceptors to regulate DA neuron firing patterns, and can thereby influence DA release throughout the brain. Although decades have passed since the discovery of somatodendritic DA release, only recently have tools become available to address fundamental questions about this process. Using intracellular application of antibodies and toxins via recording pipettes used for whole cell recording, we have shown that a given DA neuron is autoinhibited by its own DA, not by DA from its neighbors. We also find complementary roles for the intracellular Ca2 sensors, synaptotagmins 1 and 7 using this approach in wild type and knockout mice. Together, these studies reveal the primary source autoinhibitory DA, and the Ca2 sensing proteins that mediate different aspects of somatodendritic DA release.

An endogenous mechanism of action potential induction in distal dopamine axons

Changliang Liu[1], Xintong Cai[1], Andreas Ritzau-Jost[2], Paul Kramer[3], Yulong Li[4], Zayd Khaliq[3], Stefan Hallermann[2], Pascal Kaeser[1]
[1]Harvard Medical School, [2]Leipzig University, [3]National Institute of Neurological Disorders and Stroke, NIH, [4]Peking University School of Life Sciences

Information flow in neurons proceeds by integrating inputs in dendrites, generating action potentials near the soma and releasing neurotransmitters from nerve terminals in the axon. I will demonstrate that the activity of striatal cholinergic interneurons induces action potential firing in distal dopamine axons. This mechanism bypasses the law of dynamic polarization of dendrite→soma→axon information flow, serves as a physiological regulation for striatal dopamine transmission, and contributes to behavior.

Parallel Session 23 – Dopamine signalling: From intracellular pathways to striatal function and behaviour

A population-wide g-protein coupled receptor atlas of spiny projection neurons identifies novel modulators of striatal activity

Mattias Rickhag[1]
[1]Copenhagen University Hospital Amager and Hvidovre

In Parkinson's disease (PD), progressive loss of dopaminergic innervation to striatum causes an imbalance in activity of striatal projection neurons (SPNs); dSPNs become hypoactive while iSPNs become hyperactive resulting in motor deficits manifested as tremor, bradykinesia and rigidity. While the dopamine receptors, which belong to the superfamily of G protein-coupled receptors (GPCRs), have been the foremost studied biological target in PD, other GPCRs expressed in SPNs are much less characterized. We envision that yet poorly characterized striatal GPCRs, other than the dopamine D1-receptor and D2-receptor, constitute a tractable approach to restore a balanced dSPN/iSPN activity in PD. For this purpose, we have generated a cell population-wide GPCR expression atlas from SPNs by combining fluorescence-activated cell sorting using D1R-TdTomato and D2R-GFP reporter mouse lines followed by extensive quantitative PCR (qPCR) arrays. Custom-made qPCR array analysis revealed several novel GPCRs with preferential expression in either dSPNs or iSPNs. Target GPCR activation in SPNs were studied at single-neuron level by expression of genetically-encoded biosensors (calcium/protein kinase A sensor) in order to determine their modulation of SPN activity. We characterize unexplored GPCRs with strong effect to modulate activity of SPNs and demonstrate the significance of metabotropic input to SPNs. Overall, we present a comprehensive depiction of the GPCR repertoire in SPNs and identify novel modulators of striatal signaling with a therapeutic potential in PD.

Dynamics of dopamine signal integration in striatal neurons

Pierre Vincent[1], Ségolène Bompierre[1], Cédric Yapo[1], Anu Nair[2], Elia Mota[1], Jeanette Kotaleski[3], Liliana Castro[1]

[1]Sorbonne Université - CNRS, [2]KTH Royal Institute of Technology, [3]Karolinska Institutet

Striatal Medium-sized Spiny Neurons (MSNs) integrate dopamine signals through the cAMP-PKA signaling pathway. Although the signaling enzymes involved in this integration are well identified, their respective contributions to the dynamics of signal processing remain unclear. We used biosensor imaging in mouse brain slice preparations to analyze the cAMP and PKA signals triggered by transient dopamine stimulations. In silico simulations were used to test SPN's responsiveness to various dynamic dopamine signals. D1 and D2 receptors, expressed by two separate sub-classes of MSNs, showed a similar sensitivity to dopamine. The D1 response was efficiently suppressed by cholinergic agonists activating M4 muscarinic receptors, while the D2 receptor suppressed adenosine A2A signals. PDE10A appeared as the only PDE able to decrease cAMP concentration below micromolar level, and its activity was therefore required to deactivate PKA. PDE1B was shown to mediate glutamate - dopamine interactions, while PDE2A mediated a cross-talk between nitric oxide (NO) and dopamine. PDE2A and PDE4 appeared as modulators of peak dopamine responses. PKA-dependent phosphorylation appeared highly non-linear, probably as a result of DARPP-32-mediated inhibition of phosphatases. Overall, our data show that D1 MSNs are geared to respond in an all or none way to transient increases in dopamine. In contrast, D2 MSNs respond to transient lack of dopamine. Such dynamic description of signaling integration is required to better understand the effects of novel drugs, and define novel therapeutic strategies for diseases affecting the dopaminergic system.

Dopaminergic reward and performance prediction error signal are gated during courtship

Andrea C Roeser[1], Vikram Gadagkar[1], Pavel A Puzerey[1], Brian Kardon[1], Anindita Das[1], Jesse H Goldberg[1]

[1]*Columbia University*

How do social interactions affect dopaminergic (DA) responses to rewards and performance outcomes? We used electrophysiology and fiber photometry to record DA signals in two mesostriatal pathways as thirsty male songbirds sang alone and to females. When alone, singing-related performance error signals were restricted to a song-specialized mesostriatal pathway; reward prediction error signals were observed globally. When singing to a female, DA responses to both water reward and song performance outcomes were diminished and were instead driven specifically by female calls that interrupted the song. Together, we discover that reward and performance error signals are differentially routed through distinct DA pathways, that DA signals dynamically change their tuning during courtship, and that an affiliative social interaction, when precisely timed, activates distinct DA systems.

L-type channel control of DA release is gated by endogenous regulators, can we utilise them as neuroprotective strategies against Parkinson's disease?

Katherine R Brimblecombe[1], Stephanie J Cragg[1]
[1]*University of Oxford*

SNc and VTA dopamine (DA) neurons, which project to dorsal and ventral striatum respectively differ in a number of ways. Notably in their high and low sensitivity to parkinsonian degeneration respectively. We have previously identified that DA release is differentially gated by L-type voltage-gated Ca2+ channels (LTCC) in the dorsal and ventral striatum. Given that LTCC function has been identified as a stressor of DA neurons at risk for parkinsonian degeneration we are interested in identifying the molecular mechanisms regulating LTCC function. Using fast-scan cyclic voltammetry in acute ex-vivomouse brain to access mechanisms regulating LTCC control of DA release across striatal territories in both sexes. We identify calbindin-D28K as limiting LTCC function in a regionally and sexually divergent manner: D2-receptors and DA-transporters as negative and positive regulators of LTCC respectively and lastly find that targeting α2δ subunits with gabapentinoid drugs limits LTCC function without compromising DA release. Therefore LTCC-function can be dynamically and locally regulated which may prove critical for future neuroprotective strategies.

Altered intrinsic connectivity within striatal subregions is associated with anhedonia as a function of striatal tissue iron levels among youth with depression

Cecile Ladouceur[1], Teague Henry[2], Amar Ojha[1], Rasim Diler[1]
[1]*University of Pittsburgh,* [2]*University of Virginia*

Anhedonia–a core symptom of depression that leads to poor outcomes–is associated with alterations in the dopaminergic reward system, including the striatum. This study examined, among adolescents varying in levels of depression, whether resting state striatal regional homogeneity (ReHo)–an index of intrinsic regional connectivity shown to be reduced in adult depression–was associated with symptoms of anhedonia and to what extent this relationship may be moderated by striatal tissue iron, an index of dopamine(DA) function. Participants (12-17 yrs old, n=75) varying in depression symptoms completed clinical assessments and a resting state fMRI session. ReHo and mean standardized T2* (inverse proxy of tissue iron, itself a proxy for DA concentration) were calculated within the striatum. To examine the relationships between ReHo, mean T2* intensity, and anhedonia symptoms, we used a voxel-wise moderated mediation approach. Results showed that reduced ReHo was associated with higher levels of

anhedonia with higher levels of tissue iron concentration in the right caudate (peak T=4.17), and with lower levels of anhedonia in adolescents with lower levels of tissue iron concentration in the same region (peak T=2.99). Lower tissue iron concentration in the left putamen was associated with higher levels of anhedonia overall (peak T=2.79). Findings indicate that intrinsic connectivity in subregions of the striatum is associated with anhedonia but the direction of this relationship is contingent upon striatal dopaminergic function. Such findings point to the need to examine whether dopamine-targeted pharmacotherapy may be effective for a subset of adolescents with anhedonia.

Parallel Session 24 – Sex differences in dopaminergic regulation during development

Sex-specific immune mechanisms guide adolescent brain and behavioral development

Ashley Kopec[1]
[1]Albany Medical College

BACKGROUND AND AIM: Adolescence is a critical period of development for the brain's 'reward' circuitry. Synaptic pruning, or elimination, is a ubiquitous developmental mechanism crucial to neural circuit maturation, and several reports indicate that dopamine receptor pruning occurs in the reward circuitry during adolescence. In male rodents, the resident immune cells of the brain, microglia, phagocytose synapses that are 'tagged' with the immune protein complement C3, to mediate pruning during development. In this study, we thus sought to determine if microglia and C3 mediate dopamine receptor pruning in the reward circuitry during adolescence, and whether this is sex-specific. METHODS: We performed volumetric immunofluorescent reconstructions, in vivo pharmacological manipulation of the nucleus acccumbens (NAc) reward region, and social play assays. RESULTS: We determined that C3-microglial pruning regulates both NAc and social behavior development during adolescence, in a sex-specific manner. During early-mid adolescence, C3-microglial pruning decreases dopamine D1 receptors (D1rs) levels in the NAc, which in turn results in a decline in social play behavior in male rats. C3-microglial pruning also regulates female social behavior, but at a different developmental stage (pre-early adolescence) and independent of D1rs. CONCLUSIONS: These data suggest that in addition to sex-specific developmental periods and synaptic targets, neural communication underlying changes in social play during development may also be sex-specific, with males, but not females, utilizing D1rs. We predict that sex-specific developmental mechanisms may be relevant for understanding sex biases in mental health disorders that are thought to be influenced during the adolescent developmental period, including substance use disorders, schizophrenia, and depression.

A mouse model for neurodevelopmental disorders reveals male-specific alterations in repetitive behaviors driven by dopamine dysfunction

Nicola Grissom[1]
[1]University of Minnesota

BACKGROUND AND AIM: Neurodevelopmental disorders, including autism spectrum disorders, display strong male biases in diagnosis and severity, but how sex interacts with genetic factors contributing to diagnosis is unclear. Neurodevelopmental disorders comprise alterations in executive function, motivated behavior, and stereotyped behaviors, and are often treated with drugs impacting dopaminergic function. Animal models of autism-associated genetic variants show alterations in motivated behavior and stereotypies known to depend on dopamine function, and show widely-

replicated electrophysiological and molecular abnormalities in the striatum. However, little is known about how the actions of dopamine may be altered by these genotypes, or whether this could shed light on the preponderance of male diagnoses. METHODS: We have found male - specific vulnerabilities in motivated behaviors in a mouse modeling 16p11.2 hemideletion (16p11.2 del/+), one of the most frequently linked copy number variations to neurodevelopmental diagnoses including autism, as well as attention deficit/hyperactivity disorder and psychosis. We are examining the structure of behavior in these animals in locomotion and in decision making (delay discounting, risky decision making), at baseline and after treatment with amphetamine, a psychostimulant and agonist of dopamine release that is often used clinically in neurodevelopmental disorders. RESULTS: In decision making tasks, we find that external cues produce a stereotyped pattern of responding only in male carriers of 16p11.2 del/+. Acute treatment with low doses of amphetamine alter choice behaviors in wildtype males but have no impact on choices in male del/+ animals. Examining psychostimulant locomotor sensitization across multiple days of treartment, we find that doses of amphetamine that induce significant locomotion in wildtype males induce little to no broad locomotion in del/+ males, but do induce repetitive rotation. In females, there were no genotype differences in decision making or locomotion at baseline or in response to amphetamine. CONCLUSIONS These data collectively indicate that dopamine function in the striatum, potentially in the nigrostriatal pathway important to motor control and cue-driven responding, is developmentally impacted in male animals carrying this genotype, leaving females less affected. These data have implications for a number of male-biased psychological and neurological disorders implicating dopamine function, including autism, ADHD, Tourette's, and Parkinson's.

The intersection of microbiome and dopamine signaling in social dysfunction in a mouse model of prenatal stress

Caroline Smith[1]
[1]Duke University

The effects of gonadal hormones on adolescent D2 receptor-expressing medium spiny neuron excitability and behavioral modulation

Kristen Delevich[1]
[1]Washington State University

Parallel Session 25 – Dopamine beyond reward

Win-paired cues alter dopaminergic regulation of the rat gambling task

Catherine Winstanley[1]
[1]University of British Columbia

Electronic gambling machines make heavy use of win-paired cues, such as flashing lights and jingles. Theorists have begun to explore whether these sensory cues contribute to the addictive nature of these gambling products. We have shown that adding audiovisual cues, delivered concurrent with rewarding outcomes, to laboratory-based gambling tasks increases risky decision making in both rats and humans. In rats, risky decision making on the cued rat gambling task, but not the uncued version, can be

increased and decreased by acute administration of dopamine D3 agonists and antagonists respectively. Following up on this initial finding, we have since found that chronic administration of the dopamine D2/3 agonist ropinirole, which can induce gambling and impulse control disorders in a subset of patients, significantly increases risky choice when given during acquisition in the cued but not uncued task. However, all rats showed an increase in motor impulsivity while on drug, suggesting that the dopaminergic contribution to decision making and impulse control can be dissociated. Chemogenetic studies implicate dopaminergic projections from the VTA in this effect.

Lateral hypothalamic modulation of the dopamine prediction error

Melissa Sharpe[1]
[1]University of California, Los Angeles

The finding that dopamine neurons support intracranial self-stimulation (ICSS) has been taken to suggest that phasic firing of dopamine neurons is reinforcing. This is one of the last findings preventing a move away from the value hypothesis of dopamine. Yet there are almost no studies investigating the cognitive basis of ICSS. We tested how dopamine stimulation is represented in the brain during ICSS. We found that physiological frequencies did not support robust ICSS or promote behavior that would indicate the stimulation was represented as a meaningful reward in a specific or general sense. However, supraphysiological frequencies supported robust ICSS and this was associated with a representation of the stimulation as a specific sensory event that was capable of acting as a goal to motivate behavior. This demonstrates that dopamine neurons only support ICSS at supraphysiological frequencies, and in a manner that does not reflect our subjective experience with endogenous firing of dopamine neurons during learning. These data provide important considerations for the interpretation of ICSS when used to interrogate the physiological function of dopamine and other neuronal populations.

Dopamine neuron ensembles signal the content of sensory prediction errors

Erin Calipari[1]
[1]Vanderbilt University

While using associative learning frameworks to understand the neural control of behavior is powerful, these frameworks do not always account for the full range of effects of novelty on behavior and future associative learning. Here we show that dopamine in the NAc core is evoked by novel, neutral stimuli in isolation; these responses causally influence future associative learning for valenced stimuli. Using optical approaches to record and manipulate dopamine signals in the nucleus accumbens (NAc) core of awake and behaving mice, we recorded dopamine responses to neutral stimuli and defined their influence on future learned behavior. Dopamine was evoked by novel neutral stimuli and the trajectory of this response over time tracked habituation. Habituation to novel cues prior to associative learning reduced future associative learning, a psychological construct termed latent inhibition. Critically, trial-by-trial dopamine response patterns tracked this phenomenon. Finally, optically stimulating or inhibiting dopamine responses to the cue during this period bidirectionally influenced future aversive and appetitive associative learning. Our findings highlight the causal role of dopamine signaling in the NAc core in novelty-based learning in a way that cannot be predicted based on purely associative factors.

Is there a role for dopamine in aversive prediction error?

Mihaela Iordanova[1]
[1]Concordia University

BACKGROUND AND AIM: The mesolimbic dopamine signal has been linked to the key teaching signal in associative learning, prediction error. Phasic firing of dopamine neurons track prediction error about reward such that increases in firing reflect positive prediction errors and decreases negative prediction errors. Modulations in this signal in turn regulate learning about rewards and the corresponding behaviour. Advances in understanding the role of dopamine in reward prediction error have not been matched by similar investigations into the role of dopamine in aversive learning. We sought to examine the role of dopamine transients in aversive prediction error. METHODS: We used the classic blocking design, which isolates the role of prediction error in learning, in conjunction with optogenetics in the TH-Cre rat to examine how dopamine in the ventral tegmental area and its input to the nucleus accumbens regulates learning about aversive outcomes. We furthered these investigations by using a serial compound conditioning procedure to examine the role of dopamine in temporal difference learning in the aversive setting RESULTS: Our data show that stimulation of dopamine neurons or dopamine terminals in the nucleus accumbens potentiated the blocking effect in fear. Stimulation of dopamine neurons in the serial conditioning task prevented the backpropagation of conditioned fear to the earliest predictor of shock. CONCLUSIONS: Our data provide evidence for a role of dopamine in learning that goes beyond reward and extends to the aversive setting in a valence-dependent manner.

Parallel Session 26 – Common genetic and pathological drivers of dopamine dysfunction in neuropsychiatric disorders and neurogenerative diseases

Rare dopamine transporter variants in neuropsychiatric disease and parkinsonism

Freja Herborg[1]
[1]University of Copenhagen

Dopamine (DA)-related diseases include both parkinsonism and mental disorders. Although psychiatric and neurodegenerative diseases constitute mutual risk factors, little is known about the mechanisms underlying this comorbidity. The DA transporter (DAT) exerts powerful regulation of extra- and intracellular DA levels, and bi-allelic mutations in DAT is a rare cause of infantile parkinsonism-dystonia, or DAT deficiency syndrome (DTDS). By contrast, the link between insult to DAT function and mental diseases remains suggestive. We have capitalized on rare DAT variants identified in patients with DA-related disorders to study the genetic and mechanistic link between DAT dysfunction and disease. First, we have generated a novel mouse model of atypical DTDS characterized by adult disease onset and comorbid neuropsychiatric disease. Our in vivo and ex vivo analysis of these KI mice show distinct alterations in both pre-, and postsynaptic DA function, accompanied by disease-relevant behavioral alterations. Secondly, we leverage large-scale exome sequencing data from patients with mental diseases to further interrogate the genetic basis for DA transport dysfunction in psychiatric disease.

Profound effects of synuclein expression on evoked dopamine release in vivo

David Sulzer[1]
[1]Columbia University

Alpha-synuclein (a-syn) is widespread, conserved and abundant (~1% brain protein). Its presynaptic distribution suggests a role in a regulation of neurotransmitter release, but only small effects have been reported. To measure synuclein's effects on dopamine (DA) neurotransmission under physiologically relevant conditions, we developed an approach using isofluorane-anesthetized mice in which electrically-evoked burst-firing like activity (30 pulses, 50 Hz) is superimposed on native tonic activity, eliciting non-saturating levels of striatal dopamine (DA) overflow, measured by cyclic voltammetry. We found that repeated bursts at 6 minute intervals produced a fatigue (~50%) of evoked DA release in wild-type (WT) mice absent from triple knockout line (alpha, beta, gamma-synuclein:TKO). However, closely spaced bursts (6 bursts at 5 sec intervals) drove a potentiation of DA release in WT absent in TKO (~ 40% difference between WT and TKO). This potentiation appeared independent of effects on calcium in the DA axons, as GcAMP photometric signals were identical in WT and TKO, but consistent with a role for synucleins in enhanced synaptic vesicle cycling during bouts of high activity. Thus, synucleins are powerful presynaptic filters that stabilize neurotransmission during series of bursts, a feature of learning-associated activity in DA neurons.

The function of alpha-synuclein

Robert Edwards[1]
[1]University of California, San Francisco

The presynaptic protein alpha-synuclein has a central role in Parkinson's disease (PD) but like other proteins implicated in neurodegeneration, its normal function has remained poorly understood. Over-expression of synuclein, to mimick the gene multiplication that occurs in some families with PD, inhibits synaptic vesicle exocytosis, but the loss of synuclein in knockout (KO) mice has little apparent effect on synaptic transmission. To identify a role for the endogenous protein, we have studied triple KO mice lacking all three synuclein genes. Taking advantage of recent observations that synuclein can bend membranes as well as sense membrane curvature, we examined its role in the recycling of synaptic vesicles, but did not observe an effect. However, we do find a change in behavior of the pore that forms after membrane fusion. Loss of all three synuclein genes impairs dilation of the fusion pore, delaying vesicle collapse into the plasma membrane and promoting 'kiss-and-run' exocytosis. We are now using this function to test the effect of mutations associated with PD as well as modifications associated with Lewy pathology.

Dopamine dysfunction in 22q11.2 deletion syndrome: A human model for schizophrenia and Parkinson's disease

Anne Bassett[1]
[1]University of Toronto

22q11.2 deletion syndrome (22q11.2DS) is the strongest known single genetic risk factor for schizophrenia (1 in 4 individuals with 22q11.2DS develops schizophrenia). Interestingly, 22q11.2DS also

has a >20 fold-increased risk of early-onset (<45 years) Parkinson's disease (PD). While the underlying mechanisms are unclear, it could be hypothesized that a hyperdopaminergic mechanism is involved in the pathogenesis of both conditions in 22q11.2DS. First, patients with 22q11.2DS have only one copy of the catechol-O-methyltransferase (COMT) gene that is important to degradation of catecholamines. Reduced COMT expression, enzyme activity, and impaired dopamine (DA) metabolism may be the result. Indeed, increasing evidence suggests hyperDAergia in 22q11.2DS. Second, there is robust evidence for elevated striatal DA availability in patients with idiopathic psychosis. Third, autotoxicity through cytosolic DA accumulation is proposed as one of the mechanisms in the pathophysiology of PD; excessive intracellular DA outside the vesicles may be neurotoxic. Further studies are needed to determine the extent and trajectory of DA dysfunction, and relationship to clinical states, in 22q11.2DS.

Parallel Session 27 – Disentangling pre- and postsynaptic mechanisms of dopamine in reward processing

Imbalance between MSN subpopulations of the nucleus accumbens: A main feature of pathological reward processing across psychiatric disorders?

Pierre Trifilieff[1]
[1]*University of Bordeaux*

BACKGROUND AND AIM: Symptom-focused transdiagnostic approach is increasingly considered in psychiatric research in order to identify common pathological mechanisms for the improvement of treatment algorithms. In this context, alteration in reward processing is a shared symptomatic dimension of several psychiatric disorders such as major depression, bipolar disorder or schizophrenia. Numerous evidence support that this behavioral endophenotype originates from alterations within the brain reward system, however, whether the underlying pathological mechanisms are common remains largely unclear. Herein, we asked whether low n-3 polyunsaturated fatty acid (PUFA) biostatus, which has been consistently shown to be decreased in the aforementioned psychiatric disorders, could underlie reward-processing deficits. METHODS: We used dietary manipulations combined with transgenic approaches that allow alteration of PUFA levels in selective neuronal populations in mice. Reward processing was assessed through instrumental conditioning paradigms. Integrity of the mesolimbic system was studied through biochemical, neurochemical and electrophysiological approaches. RESULTS: We show that reduced n-3 PUFA biostatus in mice leads to selective impairment in the ability to maintain effort in motivational tasks. While dopamine transmission is spared in n-3 PUFA deficient animals, electrophysiological recordings reveal increased collateral inhibition of dopamine D2 receptor-expressing medium spiny neurons (MSNs) onto dopamine D1 receptor-expressing MSNs in the nucleus accumbens, a main brain region for the modulation of motivation. Such alteration of MSN network results in decreased excitability of D1-MSNs. Strikingly, transgenically preventing n-3 PUFA deficiency selectively in D2-expressing neurons normalizes MSNs collateral inhibition and enhances motivation. In contrast, the same manipulation in D1-expressing neurons, or postnatally in the whole forebrain, failed to restore motivational performance in n-3 PUFA deficient animals. CONCLUSIONS: Our data support that developmental n-3 PUFA deficiency in D2-MSN is sufficient to lead to motivational impairments in adulthood through an increase of inhibitory tone of D2-MSNs onto D1-MSNs. These results constitute the first demonstration of a causal link between a behavioral deficit and n-3 PUFA

decrease in a discrete neuronal population and suggest that lower n-3 PUFA biostatus in psychopathologies could participate to the etiology of reward-related symptoms.

Pharmacological evaluation of synthetic cathinones and their interaction with different components of the dopaminergic synapse.

Marco Niello[1]
[1]Medical University of Vienna

BACKGROUND and AIM: Synthetic cathinones are variants of known drugs of abuse developed to bypass regulations and being sold as legal alternatives to scheduled substances. Due to the variety of chemical modifications introduced by clandestine laboratories, the illicit drug markets are constantly flooded with new synthetic cathinones resulting in a rich and uncharacterized pharmacology. They can be bought online or on the street and their easy accessibility, combined with their unexplored pharmacology, often results in severe side effects such as aggression, paranoia, seizures and addiction. Synthetic cathinones are mainly targeting monoamine transporters (i.e. dopamine-, serotonin- and norepinephrine-transporter). The addictive properties of such drugs have been found associated to their selectivity towards the dopamine transporter (DAT) over the serotonin transporter (SERT). α-pyrrolidinovalerophenone (α-PVP), also known as "flakka" or "gravel", is an analog of cathinone, with a very high DAT/SERT selectivity ratio. Its potency together with its low price made it a common abused drug and often a cheap alternative to cocaine. In our work, we investigate the pharmacological profile of α-PVP enantiomers at monoamine transporters and at the more unexplored organic cation transporters by closely comparing them to cocaine. METHODS: We combined in vitro uptake inhibition assays, site directed mutagenesis, whole cell patch clamp in HEK293 cells expressing the wild-type or different mutants of the human monoamine transporters and organic cation transporters (e.g. OCT2 and OCT3), together with fast scan cyclic voltammetry (FSCV) from mouse striatal slices. RESULTS: We found differences in the enantioselectivity of α-PVP between different monoamine transporters. α-PVP displayed a remarkable enantioselectivity on both DAT and NET, but not on SERT, OCT2 and OCT3. (S)- α-PVP displayed nanomolar affinity on DAT and NET, a micromolar affinity for OCT2 and was inactive at both SERT and OCT3. Moreover, a comparison between α-PVP and cocaine binding-mode on DAT showed high similarities between the two drugs, indicating that, despite their differences in the chemical structure, their binding-site at DAT may overlap. CONCLUSIONS: Our study shows (i) pronounced differences in the enantioselectivity of α-PVP between different monoamine transporters; (ii) the involvement of OCT2 in α-PVP and cocaine pharmacology; (iii) a high degree of similarities between the binding-sites of α-PVP and cocaine at DAT. Hence, the present study provides new insight in the pharmacology of α-PVP elucidating its enantioselectivity and its similarities to cocaine. Our findings may help in developing new approaches for the treatment of psychostimulant addiction and their acute intoxication.

Differential effects of cocaine self-administration on striatal D2/3 receptor availability, psychostimulant-induced dopamine release and trait behavioral markers of drug abuse

Nathalie Ginovart[1]
[1]University of Geneva

BACKGROUND AND AIM: Current research indicates that impulsivity in rats predicts a high propensity to self-administer cocaine and is associated with low D2/3 receptor (D2/3R) levels in ventral striatum. Here, we: (i) investigated the predictive relationships between striatal D2/3R availability, evoked-dopamine (DA) release, and trait behavioral markers of drug abuse vulnerability; and (ii) determine the effect of repeated response-contingent cocaine exposure on those variables. METHODS: Roman high- (RHA) and low- (RLA) avoidance rats, which display divergent phenotypes in terms of impulsivity and novelty-seeking, were used. In a first experiment, rats (n=24-25/line) were tested for impulsivity and novelty preference using the five-choice serial reaction time task and novelty-induced place preference test, respectively. Rats were then scanned with SPECT imaging using the D2/3R radiotracer [123I]IBZM. In a second experiment, rats (n=25/line) were first tested for impulsivity and novelty preference. After a baseline SPECT scan with [123I]IBZM, rats were trained to self-administer cocaine or saline for 15 days. As a follow-up, rats were re-tested for impulsivity and novelty preference and underwent a second [123I]IBZM SPECT scan. [123I]IBZM binding was quantified to index striatal D2/3R density and amphetamine- (AMPH-) induced DA release, respectively, using a single scan and the linearized simplified reference region model. RESULTS: We found that D2/3R availability was significantly lower while AMPH-induced DA release was significantly higher in the dorsal and ventral striatum of high impulsive/high novelty-preferring (HI/HNP) RHA rats compared to low impulsive/low novelty-preferring (LI/LNP) RLA rats. Mediation analyses showed that while D2/3R availability and evoked DA release in striatum are both important predictors of impulsivity, evoked DA release fully mediates the relationship between striatal D2/3R availability and novelty preference. When exposed to cocaine, HI/HNP RHA rats exhibited higher rates of cocaine self-administration (SA) than LI/LNP RLA rats. Withdrawal from cocaine SA did not affect striatal D2/3R levels in either RHA or RLA rats, but was associated with a significant blunting of stimulated DA release in RHAs. Impulsivity was not affected in rats withdrawn from cocaine but novelty preference was selectively increased in RLA rats. CONCLUSIONS: Our data indicate that low levels of striatal D2/3R allied with an excessive presynaptic tone may represent a unique DAergic phenotype associated with high impulsivity and novelty preference and contributing to increased vulnerability to addiction. Our findings confirm the view that high impulsive action is an endophenotype predictive of vulnerability to drug use, and that the exaggerated impulsivity seen in drug abusers likely predates and is not a consequence of drug abuse. In contrast, our data suggest that the blunted release of DA observed in drug users is likely an adaptive conseq

Determinants of amphetamine-induced dopamine release in first-episode psychosis

Matthäus Willeit[1]
[1]Medical University of Vienna

BACKGROUND/AIM Patients with schizophrenia (SCZ) show a heightened behavioural response to dopamine (DA) releasing agents such as amphetamine (AMPH). Positron emission tomography (PET) studies in SCZ have shown larger AMPH-induced changes to DA D2/3 receptor radioligand binding, a marker of DA release, and increased uptake- and storage capacity of the radiolabeled DA precursor [18F]FDOPA. Aiming at further elucidating altered DA signalling in SCZ, we studied the effects of a mildly sensitizing regime of repeated AMPH administration on DA release in HV and compared it to AMPH effects in patients with first-episode psychosis (FEP) and conducted brain volumetric analyses and tested peripheral metabolic markers for their possible relationship to AMPH-induced DA release. METHODS Healthy volunteers (HV) underwent four [11C]-(+)-PHNO PET scans, one baseline PET scan without

intervention and another scan after 0.3mg /kg BW oral AMPH. AMPH was administered for two more times, after an interval of two to four weeks, no-intervention and AMPH-scans were repeated. Drug-naïve FEP patients underwent a baseline and an AMPH-scan. Data were analyzed in regions of interest and voxel-wise using the simplified reference tissue model. Volumetric parameters were derived from T1-weighted magnetic resonance images (MRI) using Freesurfer 6.0 software. Another group of HV underwent [11C]-(+)-PHNO PET scans using a bolus plus constant infusion paradigm and intravenous AMPH and [18F]FDOPA PET scans. A sub-sample was scanned again after AMPH-sensitization. Markers of lipid metabolism were derived from peripheral blood, washed erythrocytes were frozen and stored for lipidomic analyses. RESULTS Repeated AMPH amplified subcortical DA release in HV to levels observed in FEP. A vertex-wise analysis without anatomical priors showed relationships between DA release and sensitization and volumetric parameters in a left-hemispheric area corresponding to Broca's area in HV. This relationship was not detected in FEP. Analysis of peripheral lipid markers (lipidomic analysis ongoing) showed significant differences between FEP and HV in the relationship of lipid markers to [11C]-(+)-PHNO PET measures. Preliminary data support the feasibility of [11C]-(+)-PHNO bolus plus constant infusion paradigm, a preliminary analysis suggested a negative relationship between [18F]FDOPA influx and AMPH-induced DA release in the putamen of HV. CONCLUSIONS The increased AMPH-response that has been observed repeatedly in psychotic patients with SCZ can be emulated in HV by applying a mildly sensitizing regime of repeated AMPH-administration. Unless re-exposed to the drug, the neurochemical change in HV has little behavioural impact. However, as shown by differing relationships between PET markers of DA function, MRI volumetric measures and peripheral metabolic markers, our data also point towards important physiological differences between the AMPH-sensitized state and the heightened AMPH-response.

Parallel Session 28 – Selected talks from poster sessions

Leveraging CRISPR/Cas9 gene editing technologies to determine the regulators of dopamine physiology and behavior

Barbara Juarez[1]
[1]*University of Washington- Seattle*

The midbrain dopamine system is comprised of dopamine neurons originating from the ventral tegmental area projecting to downstream neural substrates that are involved in a number of behavioral domains such as reward, learning and executive function. These neurons exhibit highly coordinated tonic and phasic activity patterns and it is increasingly believed that loss of precise dopamine underlies a number of neurological and neuropsychiatric disorders. Understanding the regulators of tonic-phasic balance could lead to new insight into the underlying basis of healthy and pathological behaviors. Voltage-gated potassium channels are important regulators of neural excitability and firing. Here, we sought to elucidate how two voltage-gated potassium channel subunits thought to regulate tonic (Kv4.3) and phasic firing (KCa1.1) exert their regulatory action on the midbrain dopamine activity to modulate distinct behavioral domains. We targeted viral-based CRISPR/Cas9 mutagenesis to the coding regions of two potassium channel subunits: Kv4.3 (Kcnd3) and KCa1.1(Kcnma1) of midbrain dopamine neurons in adult DATiCre mice. Using patch clamp electrophysiology, we found that mutagenesis of Kv4.3 and KCa1.1 impart distinct neurophysiological characteristics on midbrain dopamine neurons of adult mice. We also found that these two potassium channels regulate specific behavioral domains known to be regulated by midbrain dopamine neurons such as locomotion, anxiety and social behaviors. We also

observed distinct effects on reward-associated behaviors between these two channel knockouts. These models have elucidated how dopamine neuron tonic-phasic balance contribute to healthy behavioral function in mice.

Chronic administration of D2/3 agonist ropinirole enhances the ability of win-paired cues to drive development of long-lasting preference for risky choice in a rat gambling task

Leili Mortazavi[1], Tristan Hynes[2], Catharine Winstanley[2]
[1]Stanford University, [2]University of British Columbia

Dopamine replacement therapies (DRTs) such as ropinirole are common treatments for Parkinson's Disease (PD). While effective in mitigating the motor symptoms of PD, they can induce development of impulse control and gambling disorders in a considerable proportion of patients with chronic use. To investigate the mechanisms by which DRTs precipitate these conditions, we tested the effects of chronic administration of D2/3 receptor agonist ropinirole on animal models performing a rat Gambling Task (rGT). Male Long-Evans rats (N=112) received either saline, 2.5 or 5 mg/kg/day ropinirole via subcutaneously implanted osmotic pumps over 28 days while they acquired the rGT. In this task, animals choose between four options with varying probability and magnitude of winning sucrose pellets or losing time. Half the animals were trained on a cued version of the task where delivery of rewards was paired with audiovisual cues. We found that only in the animals performing the cued rGT, administration of ropinirole, specifically during the acquisition phase of the task, biased rats towards the high-risk/high-reward options. This risk preference remained and became progressively more pronounced long after termination of drug treatment. Furthermore, consistent with previous research on the rGT, motor impulsivity was dissociable from choice effects in that it only increased transiently but returned to normal levels before the end of the drug delivery period. These findings suggest a critical role for D2/3 activity to specifically modulate the ability of win-paired cues to increase preference for risky choice. Put together with previous work, this effect may be especially powerful when options are being sampled and evaluated but not after a preference has been shaped.

Role of D2 receptor-positive ventral tegmental area dopamine neurons in effort-related motivation for food-seeking

Yoshio Iguchi[1], Shigeki Kato[1], Kazuto Kobayashi[1]
[1]Fukushima Medical University

Ventral tegmental area (VTA) dopamine neurons are critically involved in a variety of learning and motivation processes, which is reflected at least in part the phenotypic diversity of VTA neurons of which projection targets and expression molecules are distinct. We investigated how the activity of D2 receptor-positive dopamine neurons in VTA contributes to the control of the effort-related motivation of instrumental behavior reinforced by natural reward (food pellets). We developed a Drd2-Cre rat line (Nonomura et al., Neuron, 2018), and then induced the expression of the chloride ion channel derived from Caenorhabditis elegans (GluCLα/β; Lerchner et al., Neuron, 2007) in the VTA using two type of adeno-associated virus 2 (AAV2) vectors containing a double-floxed, inverted open reading frame sequence for GluCLα or GluCLβ. We reversibly inhibited the activity of the target neurons in a ligand (ivermectin) -dependent manner and examined the effects on motivation-related parameters underlying instrumental behavior estimated based on an economic demand-supply model (e.g., Mahler et al., J Neurosci., 2019). Ivermectin treatment, microinjection into the VTA as well as systemic administration, increased demand elasticity to the reward prices (number of lever presses required to obtain a unit of

reward) that we changed systematically within a training session. Ivermectin systemic administration also increased reward consumption under low-effort conditions. This result highlights the multifaceted role of the D2 receptor-positive VTA neurons in effort-related motivation for food-seeking.

Dopaminergic circuit for compulsive eating behavior

Bokyeong Kim[1], Ja-Hyun Baik[1]
[1]Korea University

Dopamine serves a central role in motivated behavior and reward processing, in which dopamine D2 receptor (D2R) is intimately involved. Palatable food drives hedonic food consumption, and hedonic drive to feed is a key contributor to compulsive eating resulting in obesity. Reduction of striatal D2R availability is observed in obese patients. The similar deficit is also detected in drug addicts, suggesting D2R is important to compulsive behavior towards the reward. We observed that D2R knockout (D2R -/-) mice consumed significantly higher amount of palatable food when limiting the access to palatable foods. In the light/dark box test, D2R -/- mice showed increased palatable food consumption in the aversive context, displaying compulsive eating behavior. It has recently been reported that the central nucleus of the amygdala (CeA) is involved in orexigenic/anorexigenic feeding behavior related to a rewards system. We previously identified D2R (+) neurons from the CeA to the bed nucleus of the stria terminalis (BNST) as a dopaminergic circuit regulating impulsivity. Selective optogenetic activation of D2R (+) neurons in the CeA→BNST circuit attenuates palatable food consumption in light/dark box test. Conversely, optogenetic inhibition increases obsessive palatable food intake. Together, these data provide evidence that D2R (+) neurons in the CeA→BNST circuit can modulate compulsive eating behavior and may be a potential therapeutic target for obesity and eating disorders. [This work was supported by the Bio & Medical Technology Development Program Grant no: 2016M3A9D5A01952412]

A mosaic of dopamine dynamics: assessing the role of dopamine neuromodulation in habit learning

Oren Princz-Lebel[1], Miguel Skirzewski[1], Claire Lemieux[2], Daniel Palmer[1], Marco Prado[1], Vania Prado[1], Penny MacDonald[1], Lisa Saksida[1], Timothy Bussey[1]
[1]Western University, [2]McMaster University

Strategies for routine behaviours, or habits, provide a rapid, efficient means for decision making, but come with a loss of behavioural flexibility. Many psychiatric and neurodegenerative disorders are characterized by aberrant decision-making and dysfunctional habit formation, including addiction and obsessive-compulsive disorder. Striatal neurocircuitry underlies the habitual control of behaviour by facilitating synaptic plasticity and strengthening stimulus-response (S-R) associations. One essential neurotransmitter that regulates activity within the striatum is dopamine, and a loss of modulatory control of striatal dopamine has been shown to impact the rate of habit formation and associated processes. However, little is known about how S-R learning is supported by fast changes in extracellular dopamine levels across different striatal subregions. Here, we uniquely combined automated touchscreen cognitive assessments, fibre photometry, and the recently developed genetically-encoded dopamine biosensor, GRABDA, to record in vivo dopamine dynamics across the dorsomedial striatum, dorsolateral striatum and nucleus accumbens while mice performed the Visuomotor Conditional Learning Task- an established cognitive task that assesses S-R learning. We show that dopamine responds dynamically during the acquisition of S-R learning, and that these response patterns differ

topographically across the striatum. Together, these findings suggest that the dopamine system in different striatal subregions plays distinct, but complementary, roles in stimulus-response learning.

A reaction diffusion model of dopaminergic and cholinergic traveling waves in the striatum
Joshua Goldberg[1], Jeffery Wickens[2]
[1]The Hebrew University of Jerusalem, [2]Okinawa Institute of Science and Technology

The maintenance of a neurochemical balance between dopamine (DA) and acetylcholine (ACh) is widely believed to be necessary for normal striatal function. However, how this balance is dynamically orchestrated is not entirely understood. To address this question, we propose an activator-inhibitor reaction-diffusion model that uses sound assumptions about the morphology of cholinergic interneuron (CIN) and DA axons and about the local, physiological coupling between them. We find that the model gives rise to self-organized coupled traveling waves of DA and CIN activation, and spatial patterns of localized "hills of activity". This simplified model is at once compatible with three recent experimental observations: 1) the dissociation between the firing of midbrain DA neurons and striatal DA levels; 2) the demonstration that CINs can activate nicotinic ACh receptors on striatal DA fibers and elicit DA release; and 3) the observation of traveling waves of striatal DA and in the CIN neuropil. We propose that the intrinsic striatal circuitry is well-suited to orchestrate the ACh-DA balance independently of midbrain DA neuron activity, with the dynamical formation of functional neurochemical striatal compartments.

Parallel Session 30 – Dopamine circuits translating motivation into action

Domain-specific dynamics and functions of striatal dopamine release in motivated behavior
Ingo Willuhn[1]
[1]University of Amsterdam

Although the cyto-architecture of the striatum is homogenous, it contains functionally heterogeneous domains that can be delineated based on their afferent projections. For example, the ventromedial striatum (VMS) receives predominantly 'limbic' afferents, the dorsomedial striatum (DMS) 'associative' input, and the dorsolateral striatum (DLS) 'sensorimotor' projections. The release of dopamine throughout the striatum plays a prominent role in basal-ganglia function and shapes motivation, movement, and reinforcement learning. However, the precise information conveyed by striatal dopamine signals, their regional specificity, and their coordination are under active debate. Thus, we characterized dopamine release in a number of distinct functional domains of the striatum of rats performing in different reinforcement-learning paradigms. Our results demonstrate substantial heterogeneity in behaviorally relevant region-specific dopamine release. Our findings identify a variety of domain-specific features of striatal dopamine signaling, demanding careful future investigation of this heterogeneous dopamine landscape, its regional function and the coordination between striatal domains.

Dopamine Signaling in the Dorsomedial Striatum Promotes Compulsive Behavior
Talia Lerner[1]
[1]Northwestern Feinberg School of Medicine

Compulsive behavior is a defining feature of disorders such as substance use disorder and obsessive-compulsive disorder. Current evidence suggests that corticostriatal circuits control the expression of established compulsions, but little is known about the mechanisms regulating the development of compulsions. We hypothesized that dopamine, a critical modulator of striatal synaptic plasticity, could control alterations in corticostriatal circuits leading to the development of compulsions (defined here as continued reward-seeking in the face of punishment). We used dual-site fiber photometry to measure dopamine axon activity in the dorsomedial striatum (DMS) and the dorsolateral striatum (DLS) as compulsions emerged. Individual variability in the speed with which compulsions emerged was predicted by DMS dopamine axon activity. Amplifying this dopamine signal accelerated animals' transitions to compulsion, whereas inhibition delayed it. In contrast, amplifying DLS dopamine signaling had no effect on the emergence of compulsions. These results establish DMS dopamine signaling as a key controller of the development of compulsive reward-seeking.

Strong and opponent contributions of dorsomedial striatal pathways to behavior depends on task demands and task strategy

Scott Bolkan[1]
[1]*Princeton University*

A classic view of the striatum holds that activity in direct and indirect pathways oppositely modulates motor output. Whether this involves direct control of movement, or reflects a cognitive process underlying movement, has remained unresolved. Here we find that strong, opponent control of behavior by the two pathways of the dorsomedial striatum (DMS) depends on the cognitive requirements of a task. Furthermore, a latent state model (a hidden markov model with generalized linear model observations) reveals that--even within a single task--the contribution of the two pathways to behavior is state-dependent. Specifically, the two pathways have large contributions in one of two states associated with a strategy of evidence accumulation, compared to a state associated with a strategy of repeating previous choices. Thus, both the demands imposed by a task, as well as the strategy that mice pursue within a task, determine whether DMS pathways provide strong and opponent control of behavior.

Parallel dopamine circuits invigorate and direct learned actions

Benjamin Saunders[1]
[1]*University of Minnesota*

Environmental cues, through Pavlovian learning, become conditioned stimuli that guide animals toward the acquisition of rewards (for example, food) by invigorating and directing seeking behavior. In previous work, I demonstrated that brief optogenetic excitation of dopamine neurons, in temporal association with visual sensory cues, can instantiate those cues as conditioned stimuli that evoke conditioned movements. Notably, I identified heterogeneous functions for dopamine neuron subpopulations in the substantia nigra (SNc) and ventral tegmental area (VTA). Investigation of the firing patterns of dopamine neurons during this process, however, showed relatively similar encoding of behavior throughout the SNc and VTA, suggesting that dopamine's functional dissociations stem from heterogeneity in signaling patterns within striatal subdomains. To investigate a circuit mechanism of this

hypothesis, my lab made use of a recently developed genetically encoded dopamine biosensor (dLight) to monitor dopamine signaling simultaneously in multiple striatal regions with fiber photometry, while movement was tracked, during Pavlovian cue conditioning. Our results demonstrate parallel activation of dopamine signaling across dorsal and ventral striatal subregions that correlates with the initiation/speed of conditioned actions, and the directed spatial pursuit of approach to conditioned cue targets, respectively. These signals evolve as learning progresses and conditioned movements become faster and more precisely targeted. Together our studies suggest that large-scale coordination of signaling across midbrain-striatal dopamine networks emerges during Pavlovian learning to orchestrate adaptive, cue-guided reward pursuit.

Wednesday May 25, 2022

Parallel Session 31 – Cannabinoid receptors and dopamine release: From reward prediction to enduring consequences

Endocannabinoid synthesis by dopamine neurons controls cue-directed reinforcement and motivation

Dan Covey[1]

[1]University of Maryland School of Medicine

Background Efficient pursuit of rewards is key to survival and relies on environmental cues and sustained motivation. However, overvaluation of certain cues or misallocation of motivational drive supports maladaptive forms of reward seeking that characterize, for example, drug abuse and obesity. Mounting clinical and preclinical work demonstrates that manipulations of endocannabinoid (eCB) signaling potently alter pathological forms of reward seeking and may represent a powerful treatment target for motivational disorders. While mesolimbic dopamine (DA) projections from the ventral tegmental area (VTA) to nucleus accumbens (NAc) control the conditioned reinforcing properties of reward-predicting cues and motivation, and are modulated by eCB manipulations, the endogenous mechanisms by which eCBs shape DA function and reward pursuit are not known. Methods To elucidate how the eCB 2-arachidonoylglycerol (2-AG) controls reinforcement and DA function, we selectively deleted the 2-AG synthesizing enzyme diacylglycerol lipase alpha (DGLa) from VTA DA neurons. DGLa deletion was accomplished by infusing an adeno associated virus (AAV) expressing Cre under the tyrosine hydroxylase (TH) promoter into the VTA of mice expressing two loxP sites flanking the DGLa gene (DGLa flox mice). Real-time DA dynamics in the NAc were monitored using fast-scan cyclic voltammetry during sucrose reinforcement. Results Conditional, targeted deletion of DGLa from VTA DA neurons disrupts the ability of predictive cues to guide reinforcement and dramatically alters how DA release in the NAc encodes cues and rewards. As control mice learn the cue-reward contingency, NAc DA release transfers from reward delivery to cue onset, a well-characterized phenomenon that adheres to classic learning theories and economic models. In contrast, DGLa deletion disrupts this pattern of DA release, such that DA predominantly responds to reward delivery and does not transfer to cue onset. Deficits in operant responding and DA dynamics further increased as the response ratio (i.e., lever presses) escalated. Alternatively, DGL? deletion has no effect when reward receipt is not reliant on cue processing and response cost is minimal. Conclusions These findings demonstrate that 2-AG mobilization from VTA DA neurons controls dopaminergic encoding of reward predictive cues and effortful responding, providing mechanistic insight into a well-established but poorly understood property of DA neurons and motivated behavior and their susceptibility to eCB manipulations.

Cannabinoid administration during adolescence: effects on anxiety and behavioural inhibition

Giovanni Hernandez[1], Armaan Fallhi[1], Dominique Nouel[1], Cecilia Flores[1]
[1]McGill University

Background and aims: Adolescence is a dynamic developmental period for the brain, particularly for the mesocorticolimbic dopamine system. Our studies have demonstrated that, during adolescence, dopamine axons continue to grow from the striatum towards the prefrontal cortex (PFC) and that this process can be disrupted by exposure to drugs of abuse and stress. These can lead to aberrant PFC function and behavioral phenotypes associated with psychopathology. This study aimed to evaluate the long-term consequences of cannabinoids given during early adolescence on (1) stress-induced anxiety-like behaviour, (2) behavioural inhibition, and (3) PFC dopamine connectivity. Methods: Male 57BL/6 mice were treated with the cannabinoid - 1/2 receptor agonist WIN-55,212-2 every other day from postnatal day (PND) 21 to PND 31. At PND60 animals were either selected for neuroanatomical analysis or exposed to Chronic Social Defeat Stress (CSDS). A separate cohort was trained in the go/no-go task upon reaching adulthood. Results: Exposure to CSDS in adulthood increased anxiety-like behavior in the elevated-plus-maze and this effect was significantly potentiated by WIN administration in adolescence. In the go/no-go task, WIN-treated mice showed dose-dependent improvement in performance; they showed faster improvement on the task from day to day and they committed significantly fewer commission errors across all days of training. At the neuroanatomical level, adolescent WIN exposure significantly increased the density of dopamine presynaptic sites in the prelimbic and infralimbic subregions of the medial PFC. Conclusions: WIN administration in adolescence potentiates anxiety-like behavior following social stress in adulthood. Surprisingly, the same adolescent treatment improves adult behavioural inhibition, which could result from increased PFC dopamine transmission.

Cannabinoid exposure in adolescence dysregulates genes that orchestrate dopamine development and cocaine-motivated behavior

Natalie Zlebnik[1], Santiago Cuesta[2], Jennifer Wenzel[1], Miguel Lujan[1], Giovanni Hernandez[3], Dominique Nouel[3], Sami Kummer[1], LanYuan Zhang[1], Cecilia Flores[3]
[1]University of Maryland School of Medicine, [2]Not listed, [3]McGill University

Cannabis is the most commonly abused illicit drug among adolescents, and excessive use in this population is associated with the development of psychiatric conditions, including drug addiction. Adolescence is a critical period for the refinement and organization of neuronal connectivity, especially within the mesocorticolimbic dopamine circuity. In particular, dysregulation of the guidance cue receptor, Dcc, in ventral tegmental area (VTA) dopamine neurons disrupts spatiotemporal targeting of dopamine axons to the nucleus accumbens (NAc) and the medial prefrontal cortex (mPFC). Here, we examine whether exposure to the synthetic cannabinoid-1/2 receptor agonist WIN-55,212-2 (WIN) in early adolescence regulates Dcc mRNA expression in the VTA and induces alterations in drug-motivated behaviors and in dopamine function in adulthood. Our findings demonstrate that adolescent exposure to WIN downregulates the Dcc receptor in the VTA and its ligand, Netrin-1, in the NAc and mPFC, suggesting disruption of pre- and postsynaptic components of mesocorticolimbic dopamine circuitry. Additionally, WIN-treated mice self-administer greater levels of cocaine accompanied by attenuated cocaine-evoked phasic dopamine transients in the NAc. Overall, these findings support that repeated

exposure to a cannabinoid-1/2 receptor agonist in adolescence impacts mesocorticolimbic dopamine system maturation and may have important implications for dopamine-mediated learning and psychostimulant-motivated behavior later in life.

Perturbation of endocannabinoid signaling and synaptic plasticity onto dopamine neurons following in utero THC

Miriam Melis[1]
[1]University of Cagliari

Cannabis use among pregnant women is increasing. Prenatal cannabis exposure (PCE) results in multifaceted molecular, cellular and synaptic adaptations that converge into a hyperdopaminergic state leading to a heightened behavioral sensitivity to acute Δ9-tetrahydrocannabinol (THC) during pre-adolescence in male rats. The objective of this study was to identify the molecular substrates contributing to the changes in synaptic properties of VTA dopamine cells from PCE male animals. We focused on the role played by the endocannabinoid (ECB) system because of its involvement in fine tuning the activity of dopamine neurons and given that THC interferes with its functions since ontogeny. We combined electrophysiological experiments in vivo and ex vivo with confocal and stochastic optical reconstruction microscopy (STORM) and quantified bassoon density measured with nanometer precision within identified axon terminals impinging on the dendrites of dopamine neurons. PCE perturbates ECB-mediated signaling at both excitatory and inhibitory synapses onto dopamine cells through specific changes in the presynaptic nanoarchitecture of these synapses. This remodeling in ECB signaling may drive changes in the filtering features of short-term synaptic plasticity and dynamically affect both excitatory and inhibitory synaptic strength onto dopamine neurons, thus contributing to the in vivo increased sensitivity to acute THC. These findings gain insights into the consequences of PCE on brain reward dopamine system function exemplified by a heightened dopamine transmission associated with the behavioral susceptibility to acute THC.

Parallel Session 32 – Dopamine regulation of inflammation and other disease processes

Dopaminergic regulation of T-cell-driven inflammation in the gut

Rodrigo Pacheco[1]
[1]Fundacion Ciencia & Vida

Evidence from inflammatory bowel diseases (IBD) patients and animal models has indicated that gut inflammation is driven by effector CD4+ T-cell, including Th1 and Th17. Conversely, regulatory T-cells (Treg) seem to be dysfunctional in IBD. Importantly, dopamine, which is abundant in the gut mucosa under homeostasis, undergoes a sharp reduction upon intestinal inflammation. Here we analysed the role of the high-affinity dopamine receptor D3 (DRD3) in gut inflammation. Our results show that Drd3-deficiency confers a stronger immunosuppressive potency to Treg, attenuating inflammatory colitis manifestation in mice. Mechanistic analyses indicated that DRD3-signalling attenuates IL-10 production and limits the acquisition of gut-tropism. Accordingly, the ex vivo transduction of wild-type Treg with a siRNA for Drd3 induced a potent therapeutic effect abolishing gut inflammation. Thus, our findings show DRD3-signalling as a major regulator of Treg upon gut inflammation.

Inflammation effects on motivation and motor function: Role of dopamine

Jennifer Felger[1]
[1]Emory University School of Medicine

Growing evidence suggests that dopamine, which can serve as an immunomodulatory factor in the periphery, is also a target for the effects of inflammation on the brain and behavior. Indeed, inflammatory cytokines have been reliably found to affect the basal ganglia and dopamine to mediate symptoms of anhedonia and motor slowing in patients with depression and in medically ill patients with fatigue, and these symptoms have been difficult to treat with standard antidepressant therapies. Translational non-human primate data from our lab showed that experimentally induced inflammation decreased availability and release of striatal dopamine, an effect that was reversed by administration of the dopamine precursor levodopa (L-DOPA). Increased endogenous levels of inflammation (plasma C-reactive protein (CRP) and cytokines) in both patients with depression and in cancer patients undergoing therapy have been associated with low functional connectivity within corticostriatal reward and motor circuits, which correlated with symptoms of anhedonia, fatigue and motor slowing. With regard to the role of dopamine, our new data in depression support the hypotheses that increasing dopamine with L-DOPA can reverse inflammation-related disruptions in corticostriatal circuitry, but only in patients with higher levels of CRP and in association with reduced anhedonia. Recent peripheral blood gene expression data further revealed that immunometabolic shifts that sustained inflammation in monocytes, as well as reduced expression of genes related to metabolism of the L-DOPA precursor, tyrosine, characterized this phenotype of depressed patients with high CRP and anhedonia. Taken together, these data in humans and non-human primates indicate that inflammation decreases dopamine synthesis and release, which has functional consequences on reward and motor circuits to drive fundamental alterations in behavior. This work suggests that development of therapeutic strategies that facilitate dopamine availability or reduce inflammation may improve symptoms of anhedonia and motor slowing in depressed or medically ill patients with increased inflammation.

Dopamine levels induced by substance use drive HIV neuropathogenesis by increasing myeloid infection and inflammation

Peter Gaskill[1]
[1]Drexel University

Current data indicate that HIV neuropathogenesis is largely driven by infection and inflammation in CNS myeloid cells, such as macrophages and microglia. Both HIV-infection and HIV-associated inflammation can be exacerbated by substance use disorders, which are highly co-morbid with HIV infection, but the specific mechanisms by which substances of abuse influence HIV neuropathogenesis is not clear. All addictive substances increase CNS dopamine concentrations, and our data suggest that the effects of dopamine on myeloid cells may be a common mechanism by which different drugs of abuse exacerbate neuropathogenesis. Specifically, we show that dopamine increases HIV infection in human primary macrophages, human microglial cell lines and iPSC-derived human microglia. This is driven by increased HIV entry into myeloid cells that are mediated by activation of a non-canonical signaling pathway that increases Ca2+ flux and PKC phosphorylation. This is mediated, at least in part, through a Gαq-activated pathway, suggesting that D1-like receptors, and potentially dopamine receptor D5 (DRD5), are the

primary receptors initiating this signaling. Dopamine signaling in these cells also increases expression of the chemokine receptor CCR5, a viral co-receptor, on the cell surface and promotes an inflammatory phenotype in these cells. Myeloid cells exposed to levels of dopamine induced by substances of abuse show increased activation of NF-□B and the NRLP3 inflammasome, resulting in increased production of inflammatory mediators such as IL-6, IL-1β and CXCL-10, as well as priming the NLRP3 inflammasome. Overall, these data show that increases in dopamine can enhance both viral replication and inflammation, potentially increasing the size of the viral reservoir and promoting further tissue damage in the brain. This indicates that dopamine may be a significant factor in the progression of HIV-neuropathogenesis in individuals with substance use disorders or those using therapeutics that modulate the dopaminergic system.

Functional characterization of the biogenic amine transporters on human macrophages

Habibeh Khoshbouei[1]
[1]University of Florida

Monocyte-derived macrophages are key players in tissue homeostasis and diseases regulated by a variety of signaling molecules. Recent literature has highlighted the ability for biogenic amines to regulate macrophage functions, but the mechanisms governing biogenic amine signaling in and around immune cells remains nebulous. In the central nervous system (CNS), biogenic amine transporters are regarded as the master regulators of neurotransmitter signaling. While we and others have shown that macrophages express these transporters, relatively little is known of their function in these cells. To address these knowledge gaps, we investigated the function of norepinephrine (NET) and dopamine (DAT) transporters on human monocyte-derived macrophages. We found that both NET and DAT are present and can uptake substrate from the extracellular space at baseline. Not only was DAT expressed in cultured monocyte-derived macrophages (MDMs), but it was also detected in a subset of intestinal macrophages in situ. Surprisingly, we discovered a NET-independent, DAT-mediated immuno-modulatory mechanism in response to lipopolysaccharide (LPS). LPS induced reverse transport of dopamine through DAT, engaging an autocrine/paracrine signaling loop that regulated the macrophage response. Removing this signaling loop enhanced the pro-inflammatory response to LPS. Collectively, our data introduce a potential role for DAT in the regulation of innate immunity.

Parallel Session 33 – Multimodal GPCR actions regulate brain dopamine function

Designing Bitopic Molecules for Dopamine D2-like Receptors: The Whole is Greater Than the Sum of its Parts

Amy Newman[1]
[1]National Institute on Drug Abuse

The strategy of designing bivalent or bitopic molecules that engender unique pharmacological properties has evolved as an attractive way to engineer highly selective compounds for targeted G-protein coupled receptors (GPCRs) with optimized efficacies and/or signaling bias. The emergence of X-ray crystal structures of many GPCRs, including all three D2-like receptor subtypes, and the identification of both orthosteric and allosteric binding sites have provided further guidance to bitopic ligand drug design that includes a primary pharmacophore (PP), a secondary pharmacophore (SP) and a linker

between them. We have strategically designed combinations of these three components to create highly D3 receptor (D3R) selective agonists, antagonists and partial agonists, and have also discovered compounds that appear to show functional bias, in vitro. Computational studies demonstrate that the PP, SP and linker all play critical roles in drug-receptor interactions, at the protein level. Nevertheless, identifying optimal pharmacological and drug-like profiles for development as pharmacotherapeutics remains a challenge, especially for the treatment of neuropsychiatric disorders. Our most recent D3R-selective partial agonists for the treatment of substance use disorders will be highlighted.

Developmental regulation of prefrontal fast-spiking interneurons by D2 receptor-β-arrestin signaling

Kuei Tseng[1]
[1]University of Illinois

Proper maturation of the prefrontal cortex (PFC) during adolescence is critical for the acquisition of cognitive control. Disruptions of these functions are often found in psychiatric disorders that emerge during adolescence. However, the neurobiology underlying this adolescent vulnerability remains unclear. Of particular interest is the role of dopamine and its regulation of PFC excitability during adolescence. The recent development of functionally selective dopamine receptor ligands targeting G protein or β-arrestin signaling provide a new tool to study how dopamine-mediated signaling is developmental regulated in the PFC. Here I will discuss how D2R-β-arrestin signaling may contribute to prefrontal maturation during adolescence through its excitatory action onto local fast-spiking interneurons. Interestingly, the impact of the D2R-β-arrestin ligand is cell-type specific and distinct from the effects observed with G protein biased ligands or following activation of D4R-β-arrestin signaling. These results indicate that D2R and D4R biased ligands could be used to reveal how distinct GABAergic cells in the PFC are modulated by dopamine during adolescence. As fine-tuning of PFC output is highly dependent on local GABAergic function, a disruption of D2R-β-arrestin signaling is expected to elicit enduring PFC-dependent cognitive deficits. remains unclear. Of particular interest is the role of dopamine and its regulation of PFC excitability during adolescence. ? METHODS: The recent development of functionally selective dopamine receptor ligands targeting G protein or β-arrestin signaling provide a new tool to study how dopamine-mediated signaling is developmental regulated in the PFC. ? RESULTS: Here I will discuss how D2R-β-arrestin signaling may contribute to prefrontal maturation during adolescence through its excitatory action onto local fast-spiking interneurons. Interestingly, the impact of the D2R-β-arrestin ligand is cell-type specific and distinct from the effects observed with G protein biased ligands or following activation of D4R-β-arrestin signaling. ? CONCLUSIONS: These results indicate that D2R and D4R biased ligands could be used to reveal how distinct GABAergic cells in the PFC are modulated by dopamine during adolescence. As fine-tuning of PFC output is highly dependent on local GABAergic function, a disruption of D2R-β-arrestin signaling is expected to elicit enduring PFC-dependent cognitive deficits.

Discovery and characterization of a functionally selective ghrelin receptor ligand for modulating brain dopamine homeostasis

Joshua Gross[1]
[1]Duke University

The growth hormone secretagogue receptor-1a (GHSR1a) is the cognate G protein-coupled receptor (GPCR) for the hormone ghrelin. The GHSR1a is a modulator of brain dopamine (DA) homeostasis and neuroprotective within DA neurocircuits. GHSR1a-mediated signaling originates from pharmacologically separable G protein- and β-arrestin (βarr)-dependent pathways and consequently, GHSR1a-dependent physiological responses rely upon their distinctive signaling contributions. Thus, when treating disorders of disrupted DA homeostasis, a pharmacological strategy that modulates biased GHSR1a signaling may uncouple desired therapeutic outcomes from unwanted side effects. By high-throughput screening of ~47,000 small molecules, we discovered a GHSR1a-selective, G protein-biased agonist — N8279 (NCATS-SM8864) — based on a novel chemotype. Comprehensive pharmacological characterization reveals that N8279 elicits potent and biased G□q activity at both the apo- and ghrelin-bound GHSR1a. Further biochemical analysis and molecular modeling demonstrate that N8279 signaling requires sites within the extracellular domain of the GHSR1a, especially extracellular loop 2 (ECL2). Collectively, our findings support that N8279 possesses a bitopic, extended binding mode into the GHSR1a ECD that preferentially favors G□q signaling over alternative G proteins (G□i/o, G□12/13) and □arr2-dependent cellular responses. Critically, N8279 is brain penetrant in mice and attenuates dysfunctional DA-mediated behaviors in both genetic and pharmacological mouse models of hyperdopaminergia. Our findings provide insight into mechanisms governing GPCR signaling and illustrate how functional selectivity can be leveraged to develop GHSR1a pharmacotherapeutics that normalize pathological disruptions of brain DA homeostasis.

Selective Modulation of Dopamine-Associated Behaviors by a Biased Allosteric Modulator of Neurotensin Receptor 1

Lauren Slosky[1]
University of Minnesota

Neurotensin receptor 1 (NTSR1) is an endogenous modulator of brain dopamine signaling. Small molecule NTSR1 agonists have been pursued for more than 40 years as potential therapeutics for dopamine-associated psychiatric disorders, including psychostimulant addictions. While such compounds remained elusive, characterization of NTSR1's diverse physiological effects made apparent that desired dopamine neuromodulatory action may be accompanied by unwanted effects, including hypotension and hypothermia. As a G protein-coupled receptor (GPCR), NTSR1 signals through the canonical activation of G proteins and engages β-arrestins to mediate distinct cellular signaling events. We have now developed an allosteric NTSR1 modulator, SBI-553, that not only exhibits β-arrestin activity on its own, but also extends profound β-arrestin bias to the endogenous ligand by selectively antagonizing G protein signaling. SBI-553 shows efficacy in animal models of psychostimulant abuse without the side effects characteristic of balanced NTSR1 agonism. These findings indicate that the dopamine neuromodulatory action of NTSR1 may be selectively preserved in β-arrestin biased ligands, thus providing a strategy to develop safer NTSR1-targeting anti-addiction therapeutics with more directed pharmacological action.

Parallel Session 34 – Unusual suspects in dopamine and dopaminoceptive systems

Unraveling a novel striatal output opposing the classical D1-direct and D2-indirect bimodal model.

Bruno Giros[1]
[1]McGill University

Patricia Bonnavion*, Quentin Rainer*, Christophe Varin, Aurélie Degroote, Elisa Pozuelo, Amandine Cornil, Kathleen Xu, Elsa Isingrini, Sylvie Dumas, Alban De Kerchove#, Bruno Giros#. *: co-first authors #: co-last authors Present in only a few thousand neurons in the rodent brain, dopamine (DA) is a major regulator of sensory-motor and cognitive functions. In mammals, the largest number of DA cell bodies is located in the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc): two tegmental structures that project to the basal ganglia, limbic, and cortical formations. The importance of DA neurotransmission is emphasized by its direct implication in neurological and psychiatric disorders. DA control of psychomotor functions essentially arises from its complex regulatory role on the thalamo-cortico-striatal loop. The architectural organization of striatal output into a direct and an indirect pathway early emerges from anatomical and functional studies. The direct pathway originates from GABAergic medium spiny neurons (MSN) containing substance P, dynorphin and the dopamine receptor D1R, and it projects to the internal globus pallidus and the substantia nigra pars reticulata (GPi/SNr). The indirect pathway arises from MSNs containing enkephalin, the adenosine receptor 2a and the D2R, and it projects to the GPi/SNr through sequential connections to the external segment (GPe) of the GP and the subthalamic nucleus (STN). Activation of neurons in the direct and indirect pathways has opposite output effects on the thalamo-cortical loop, respectively disinhibiting and dis-disinhibiting the thalamo-cortical section of the loop. However, because DA stimulates MSNs in the direct pathway through dopamine D1R activation, while it inhibits MSNs in the indirect pathway through dopamine D2R activation, its overall action synergizes the thalamo-cortical loop activation. Our central hypothesis is that a neuronal sub-population of striatal Medium Spiny Neurons has an opposing functional role on the Striatal-Nigra-Thalamo-Cortical loop than these synergizing D1-and D2-MSNs. We will present the development of unique new animal models and molecular tools and strong experimental evidences pointing to a revisited model of the striatal output.

Cue-evoked dopamine promotes conditioned responses during learning

Sebastian Haesler[1]
[1]KU Leuven & Neuroelectronics Research Flanders (NERF)

Joachim Morrens, Cagatay Aydin, Aliza Janse van Rensburg, José Esquivelzeta Rabell and Sebastian Haesler VIB, 3001 Leuven, Belgium ; Imec, 3001, Belgium ; KU Leuven, Department of Neuroscience, Research Group Neurophysiology, 3000, Leuven, Belgium ; Neuroelectronics Research Flanders, 3001 Leuven, Belgium Dopamine neurons play a key role in associative learning. They mediate the association of conditioned stimuli (CS) with reward (unconditioned stimuli, US) by signaling the discrepancy between predicted and actual reward during the US, i.e. reward prediction errors (RPE, Schultz et al., 1997). RPEs play a central role in formal theories of reinforcement learning (Mackintosh, 1975; Pearce and Hall, 1980; Rescorla and Wagner, 1972; Sutton and Barto, 1998) and experimental manipulations of dopamine with optogenetics, specifically during the US period have confirmed that dopamine RPE responses causally impact associative learning (Chang et al., 2016; Steinberg et al., 2013). According to variants of RPE-centered frameworks, associative learning is also directly influenced by the CS through

variations of its salience or associability (Mackintosh, 1975; Pearce and Hall, 1980). Associability refers to the potential of a stimulus to be associated with another stimulus through learning. The concept of CS associability has also provided the cornerstone for models of latent inhibition, i.e. the common behavioral observation that conditioned responses to familiar cues establish much slower during associative learning than those to novel cues (Lubow, 1989; Lubow and Moore, 1959). According to CS associability frameworks, experiencing stimuli which are not followed by an event or consequence reduces their associability, resulting in latent inhibition. Pharmacological evidence has implicated the dopamine system in latent inhibition but the circuit mechanism by which the activity of dopamine neurons relates to the different learning rates of novel and familiar stimuli remains unknown today. Here, we used fiber photometry to characterize dopamine responses to inconsequential familiar and novel stimuli. Using bidirectional optogenetic modulation during conditioning, we then show that CS-evoked dopamine promotes conditioned responses. Our results thus provide direct experimental support for CS associability models and suggest, associative learning is influenced by CS dopamine in addition to US reward prediction errors. Accordingly, the absence of dopamine responses to familiar CS might explain their slower learning in latent inhibition.

The Modulation of Excitation and Inhibition by Cocaine and Neuropeptides in the Ventral pallidum is Cell Type Specific

Daniela Neuhofer[1]
[1]Medical University of South Carolina

Daniela Neuhofer and Peter Kalivas The ventral pallidum (VP) is an integral component of the reward circuitry and is a major target of GABAergic innervation of both D1 Medium spiny Neurons (MSNs) and D2 MSN from the nucleus accumbens. Although the majority of VP neurons are GABAergic (VPGABA), the VP also contains a significant population of glutamatergic (VPGlu) neurons. It was recently demonstrated that VPGABA drive positive reinforcement, whereas VPGlu drive behavioral avoidance. Also MSN afferents in the VP exert opponent control over behavioral reinforcement with activation of D1 MSN afferents promoting and D2 afferents inhibiting reward seeking. Although the innervation pattern onto VP neurons cannot conclusively explain behavioral output, connectivity pattern could be shifted via experience dependent synaptic plasticity. In addition to being segregated according to the expression of D1 versus D2 receptors, the two subpopulations of MSNs segregate according to co-expressed neuropeptides, with D1 MSNs expressing substance P and dynorphin (activating NK1 receptors and kappa opioid receptors respectively), and D2 MSNs co-expressing enkephalin and neurotensin (activating mu opioid receptors and neurotensin receptors respectively). Earlier studies show that administration of these neuropeptides or analogues into the VP alters appetitive behavior and cocaine seeking. We aimed to investigate how D1 and D2 inputs onto VPGlu and VPGABA are modulated before and after extinction from cocaine self-administration. Using whole cell patch clamp recordings in Ai6::VPGlu and Ai6::VPGABA reporter mice, we describe a weakening of GABAergic synapses onto VPGABA and a strengthening onto VPGlu after cocaine self-administration. To test whether also neuropeptides differentially modulate VPGlu and VPGAT Neurons, we quantified the relative strength of excitatory and inhibitory inputs (E/I Ratio) onto VPGlu and VPGAT Neurons and how this E/I Ratio changed after pharmacological activation of neuropeptidergic target receptors in the VP. We found that activation of NK1R does not affect E/I ratio in VPGAT Neurons but decreases E/I ratios in VPGlu neurons via inhibition of glutamate transmission. Activation of MOR increase E/I ratios in VPGlu

via inhibition of GABA transmission but no clear effect on VPGAT neurons. Our results can provide a circuit-based model to explain the antagonistic effect of D1 and D2 MSN afferents in the VP on reward behavior.

Addiction elsewhere than in dopaminergic neurons

Alban de Kerchove d'Exaerde[1]
[1]*Université Libre de Bruxelles*

Motivational processes are under the critical influence of the ventral part of basal ganglia, comprising several interconnected nuclei (as striatum, globus pallidus and ventral tegmental area (VTA)). Addictive drugs increase extracellular dopamine (DA) levels in the ventral striatum, Nucleus Accumbens (NAc), and share this ability despite varied pharmacological properties and mechanisms of action. A major goal in the field of drug addiction has been to uncover the molecular, cellular and circuit mechanisms underlying addiction-associated neuroadaptations. It has been hypothesized that one such mechanism is the regulation of gene expression, and there have been numerous studies that have documented altered expression of genes in the NAc after administration of addictive drugs. We discovered that Maged1 (Melanoma antigen genes d1) has a mandatory role in neurochemical, functional and behavioural changes related to drug addiction. Mice lacking Maged1 have an impairment of DA release in NAc after cocaine injection and are insensitive to the behavioural effects of cocaine as assessed by locomotor sensitization, conditioned place preference (CPP), and drug self-administration. Electrophysiological experiments in brain slices and conditional KO mice demonstrated that Maged1 is critical for cortico-accumbal neurotransmission. Further, to understand the mechanism of action of Maged1 we inactivated Maged1 in specific neuronal populations and demonstrated that the effects of Maged1 knockout are independent of its expression in DA and GABA neurons. However, expression of Maged1 in prefrontal cortex, amygdala or ventral hippocampus, is required for cocaine-induced extracellular DA release in the NAc as well as cocaine-mediated behavioural sensitization and acute cocaine effect . As these 3 nuclei are mainly glutamatergic (Glu) we are currently determining which Glu neuronal populations are more precisely involved. In addition, we are performing RNAseq of cell sorted neurons to identify the genes targeted by Maged1. This work identifies Maged1 as a critical molecule in Glu neurons affecting DA release and behavioural models of addiction.

Parallel Session 35 – Selected talks from poster sessions

Illuminating dopamine dynamics in Huntington's disease

Sarah Yang[1], Markita Landry[1], David Schaffer[1]
[1]*University of California, Berkeley*

Dysregulation of dopamine transmission plays a key role in multiple neurodegenerative diseases. In Huntington's Disease (HD) this dysregulation is thought to be biphasic, with increases in dopamine levels accompanying early chorea and decreases accompanying late akinesia. While several treatments for physical and psychiatric HD symptoms target dopaminergic neuromodulation, little is known about the relationship between dopamine and the principal cause of HD, production of mutant huntingtin protein. This lack of understanding is partly due to limited capability to visualize dopamine dynamics at the spatiotemporal resolution of both neuromodulator release (ms) and boutons (µm). Prior studies

measuring bulk-averages of dopamine release suggest that evoked release in the dorsal lateral striatum decreases in R6/2 HD mice and coincides with motor symptom onset. However, knowledge of what drives decreased dopamine release is uncertain and could encompass decreased dopamine release sites, decreased dopamine quantal release per site, or a combination of the two. Herein, we utilize a near Infrared Catecholamine nanosensor (nIRCat), to image "hot spots" of dopamine activity in the striatum of R6/2 HD mice and find that late-disease decreases in evoked dopamine release are primarily driven by decreases in the number of hot spots as opposed to decreasing dopamine release quanta. These findings motivate investigation into how dopaminergic projections are affected by mutant huntingtin and whether specific targeting of these loci is important for developing gene and cell therapy efforts.

A novel target for neuroprotection: The small GTPase Rin inhibits LRRK2 to promote autophagy and reduce alpha-synuclein pathology

Anne-Marie Castonguay[1], Julia Obergasteiger[1], Mattia Volta[2], Martin Lévesque[1]
[1]Laval University, [2]Eurac Research

Parkinson's disease (PD) is characterized by the accumulation of misfolded alpha-synuclein (aSyn) in the substantia nigra (SNc), leading to the death of dopaminergic (DA) neurons. The mechanisms underlying aSyn pathology are still unclear but hypothesized to involve autophagy and endosome-lysosome pathways (ALP). LRRK2 mutations are a major cause of familial and sporadic PD. Pharmacological inhibition of LRRK2 kinase activity ameliorates ALP deficits and reduces pS129-aSyn inclusions, indicating that these phenotypes depend on LRRK2 hyperactivation. We observed selective down-regulation of the novel PD risk factor RIT2 in LRRK2 mutant cells (G2019S). RIT2 encodes the small GTPase Rin, which is enriched in DA neurons and reduced in the SNc of PD brains. We aim to evaluate if Rin can modulate LRRK2 kinase activity to rescue alterations in autophagy and promote aSyn clearance. Rin overexpression in LRRK2-G2019S neuroblastoma cells rescued the alterations in ALP and diminished aSyn inclusions. In vivo, viral mediated overexpression of Rin prevented motor deficits induced by AAV-A53T-aSyn injection. Overexpression of Rin also protected against the loss of dopaminergic axons in the striatum and neuronal degeneration in the SNc. Furthermore, RIT2 overexpression prevented the A53T-aSyn-dependent increase of LRRK2 kinase activity in vivo. On the other hand, reduction of RIT2 levels leads to defects in the ALP, similar to the ones induced by the G2019S LRRK2 mutation. Our data indicate that RIT2 is required for correct lysosome function, inhibits overactive LRRK2 to ameliorate ALP impairments and counteract aSyn aggregation and related deficits. Targeting RIT2 could thus represent a novel strategy to combat neuropathology in familial and idiopathic PD.

Early activation of dopaminergic system alters behavior and neural branching of prepubertal mice in a sexually dimorphic manner

Laila Arabe[1], Muiara Moraes[1], Ana Luiza L. Reis[1], Bruna Resende[1], Sofia Avritzer[1], Paula Valverde[1], Bruno Souza[1]
[1]Universidade Federal de Minad Gerais

It is known that some neuropsychiatric disorders share both dopaminergic (DA) and neurodevelopmental hypothesis. However, little is known about the role of DA signaling in the dynamic of brain development. The first 5 postnatal days (PD) is a developmental window on which there is a high rate of synaptogenesis. Thus, we investigated if DA imbalance during these first 5 PD affects the behavior of mice before puberty. For this, we daily i.p. treated newborn mice with L-Dopa Benserazide,

D2 receptor agonist Quinpirole (Qui), D1 receptor agonist SKF-38393 or saline (Sa) from PD1 until PD5. At the age of 30 days, we evaluated exploratory behavior, anxious- and depression-like behavior by open field test (OF), elevated plus maze (EPM), novelty suppressed feeding test (NT) and forced swim test (FST). In addition, we investigated the hippocampal neuronal branching by Golgi-cox method. Females treated with SKF showed a decrease in the number of head dippings on EPM, an increase of the latency time on NT, and a reduction in the hippocampal neuronal branching. Males treated with SKF showed a decrease in the number of stretching on EPM and an increase in the immobility time on FST. Males treated with Qui showed a decrease in distance walked on OF and in climbing time on EPM. In order to investigate if DA signaling is altered on prepubertal mice, we i.p. treated L-dopa Benserazide 30 minutes before behavioral tests. The mice previously treated with L-Dopa during the first 5 PD showed higher behavioral response to the new administration with L-Dopa on OF and FST compared to those previously treated with Sa. Our results suggest a sexual dimorphic function of DA during the early postnatal brain development, affecting prepubertal behavior, brain morphology and dopaminergic signaling.

Axon-derived netrin-1 regulates midbrain GABAergic migration and substantia nigra development

Divya Darwin Arulseeli[1], Sara Brignani[1], Ewoud Schmidt[1], Ozge Dudukcu[1], Laurens Grossouw[1], Youri Adolfs[1], Juan Moreno-Bravo[2], Alain Chedotal[2], Jeroen Pasterkamp[1]
[1]Utrecht University, [2]Sorbonne Université

Cell migration in the central nervous system is shown to be regulated by guidance cues produced locally by cells residing at choice points. We show a hitherto unknown role for Netrin-1 in the migration of dopaminergic neurons and positioning of GABAergic neurons for the formation of Substantia nigra pars reticulata (SNr). Mice lacking Netrin-1 protein show increased lateral-ventral migration of dopaminergic neurons of the compacta (SNc). Normally, SNc neurons project their dendrites ventrally into the GABA-rich SNr. In Netrin-1 KO mice, GABAergic neurons of the anterior SNr fail to migrate ventrally to form this brain nucleus. Intriguingly, conditional ablation of Netrin-1 from local cellular sources in the midbrain does not mimic the defects in cellular migration seen in the complete knockout. Alternatively, we find that Netrin-1 derived from forebrain neuronal axons affects migration and positioning of neurons in the SNc/SNr in the ventral midbrain. We also identify the possible Netrin-1 signaling receptor that mediates these effects of Netrin-1. This study signifies the role of axons as "carriers" of guidance cues to affect cellular organization and connectivity in the brain.

Dopamine spatiotemporal dynamics comparison between members of the dLight sensors family

Julie Chouinard[1], Akash Pal[2], Sakiko Takahashi[1], Kiyoto Kurima[1], Nobuyoshi Kitamura[1], Lin Tian[2], Jeffery Wickens[1]
[1]Okinawa Institute of Science and Technology, [2]University of California Davis

The growing toolkit of genetically encoded fluorescence biosensors opens new avenues for studying the spatiotemporal dynamics of neurochemical signaling in the brain. These sensors directly report synaptic release with specificity and high resolution. dLight1 is a family of dopamine (DA) sensors based on three inert human DA receptors with broadly tuned apparent affinity and dynamic range suitable to measure DA release between pM and µM range. However, the intrinsic properties of sensors such as expression level and patterns, apparent affinity, kinetics, and dynamic range, can profoundly affect signal-to-noise ratio (SNR) for in vivo applications that is brain-region and cell-type specific. We developed a viral-based strategy to maximize the SNR of DA imaging in various brain regions. We reported an optimized dLight,

dLight2.1, with improved SNR and compared its performance with dLight1.3b and RdLight driven by various viral vectors in both mPFC and striatum. We also compared the performance of DA imaging with simultaneously recorded fast-scan voltammetry traces with electrical stimulation with and without the DA reuptake inhibitor methylphenidate. When sensors are expressed perisynaptically, they appear to report dopamine levels near release sites, whereas voltammetry detects DA that has diffused some distance. A comparison between sensors revealed differences in dopamine levels and spatiotemporal dynamics that are dependent on expression level and pattern. Our data show that dLight sensors can pave the way toward a more complete understanding of neurotransmitter dynamics in the basal ganglia circuitry and beyond. With improved imaging and analysis methods, these biosensors could be useful tools to decipher neural activity into its composite molecular signaling events.

Cardio-Metabolic and Psychiatric Comorbidities: Early Adversity- Mesocorticolimbic Dopamine Gene Network Interactions

Barbara Barth[1], Danusa Mar Arcego[1], Euclides De Mendonça Filho[2], Randriely Merscher Sobreira de Lima[2], Irina Pokhvisneva[3], Zihan Wang[1], Michael Meaney[1], Patricia Pelufo Silveira[1]
[1]McGill University, [2]Universidade Federal do Rio Grande do Sul, [3]Ludmere Centre

Psychiatric disorders are commonly comorbid with cardio-metabolic conditions through largely unknown biological pathways. We hypothesized that early life adversity would functionally link these conditions with the mesocorticolimbic dopamine system as a critical moderator pathway. We tested this hypothesis using a co-expression based polygenic score (ePRS) reflecting variations in the function of the dopamine transporter (DAT) gene network in the prefrontal cortex and striatum, the final targets of the mesocorticolimbic pathway. As predicted, the mesocorticolimbic DAT1 ePRS significantly moderated the impact of early life adversity on the risk for both psychiatric (schizophrenia, neuroticism, mood and substance use disorders) and cardio-metabolic (type 2 diabetes, atherosclerosis, cardiovascular disease) comorbidities in adults (UK Biobank, N= 60016) and adolescents (ALSPAC, N= 910). Brain gray matter densities in the insula and prefrontal cortex were significantly associated with SNPs from the DAT1 ePRS implicating these regions as critical dopaminergic targets for psychiatric/cardio-metabolic comorbidities. These results reveal that psychiatric and cardio-metabolic comorbidities share common developmental pathways and underlying biological mechanisms.

Parallel Session 36 – Guys and dolls: Sex effects in dopamine genetics, circuits and drug action

Sexually dimorphic dopamine signaling dictates the penetrance and behavioral trajectory of human dopamine transporter coding variation

Adele Stewart[1]
[1]Florida Atlantic University

The dopamine transporter (DAT) Val559 variant, identified in patients with ADHD, bipolar disorder, and autism spectrum disorder, supports non-vesicular dopamine leak we termed anomalous dopamine efflux (ADE) and leads to homeostatic dopamine disruptions in mice harboring the mutation. We now report profound, sex-dependent phenotypic divergence in DAT Val559 mice that derives, at least in part, from circuit-specific dopamine D2 receptor (D2AR)-DAT coupling. In male DAT Val559 mice, presynaptic nigrostriatal D2ARs are constitutively-activated, driving enhanced DAT surface trafficking and phosphorylation, an effect is absent in DAT Val559 females. However, females show constitutive D2AR

activation of presynaptic mesolimbic D2ARs. In parallel we have identified region-dependent, sex-specific differences in DA clearance and sexually dimorphic behaviors both at baseline and in response to DAT-targeted drugs. Our work provides a cogent example of how a shared biological insult drives alternative physiological and behavioral trajectories due to a sex biased neuronal architecture.

Interactions between synaptic zinc, dopamine, and cocaine as a function of sex

Oscar Solis[1]
[1]*National Institute of Drug Abuse*

Glutamatergic signaling in the striatum is implicated in cocaine use disorder. Within a subset of glutamatergic neurons, vesicular zinc (Zn2+) is co-released with glutamate and modulates dopamine transmission. Previous studies demonstrated that estrogen reduces Zn2+ in the brain and that Zn2+ exerts sexually dimorphic effects on locomotion and at skilled motor learning tasks. Recently, we found that cocaine increases Zn2+ levels in the striatum, and that Zn2+ increases the in vivo potency of cocaine by binding to the dopamine transporter (DAT). In line with these findings, we showed that the absence of vesicular Zn2+ induces a decrease in conditioned place preference, locomotor sensitization and self-administration to cocaine. In this talk I will present data describing interactions between sex, Zn2+, and DAT in relation to cocaine exposure.

Sex-specific behavioral strategies to elucidate neurobiology of adaptive decision-making

Jennifer Zachry[1], M.G. Kutlu[1], Erin S. Calipari[1]
[1]*Vanderbilt University*

In recent years, research focused on understanding the biological variables contributing to psychiatric disorders has highlighted sex-based differences in the development and presentation of symptoms as well as in fundamental behavioral processes. A large body of work has focused on understanding stimulus-driven behavior, sex differences in these processes, and the neural circuits underlying them. Sex-based differences in reward seeking and avoidance offer an ideal model to explore bias and strategy in a behavioral task while providing insight into the neurobiological basis of information encoding. For example, even though females will self-administer opiates at higher rates than males, when given a choice between opiates and a high-fat reward they choose the non-drug reinforcer over the drug alternative at a higher rate than males, clearly highlighting that sex differences do not manifest themselves as universal behavioral principles, but rather are a complex interaction between sex and environment. Further, work in rats has shown while females are more motivated to self-administer drug and non-drug rewards, they are also more sensitive to punishment. Numerous other studies have varied the magnitude, value, and probability of rewards highlighting that females are more risk averse than males. Capturing this complexity necessitates behavioral tasks that can probe the balance in the subjective value of rewarding versus aversive stimuli and their antecedent cues. We established a task—the Multidimensional Cue Outcome Action Task (MCOAT)—that allows for quantitative assessment of multidimensional behavioral functions relevant to human decision making in mice. By combining negative reinforcement, punishment, and positive reinforcement we can dissociate action from stimulus valence. First, we identified sex-specific behavioral strategies, showing that females prioritize avoidance of negative outcomes over seeking positive, while males have the opposite strategy. Next, we showed that while males were more sensitive than females to unsignaled shocks of varying intensities, as measured by motor response and vocalizations, females are more sensitive to punishment. Our findings suggest male mice are more sensitive to lower shock intensities, while females are more sensitive to the

effects of shock on reinforcement/punishment. Lastly, in order to understand how real-time activity in these populations is linked to behavioral execution, we expressed the genetically encoded calcium indicator (GCaMP6f) within D1 and D2 MSNs. We coupled this calcium indicator with in vivo fiber photometry and single-photon cellular resolution calcium imaging to record from these cell populations in awake and behaving animals to identify correlated changes in the calcium signal that relate to behavioral differences in male and female mice.

Sex and menopause influence dopaminergic neuronal vulnerability in a novel AAV-SNCA mouse model of Parkinson's disease

Roberta Marongiu[1]
[1]*Cornell University*

Parkinson's disease (PD) is one of the most common neurological disorders with a higher prevalence in men than women. While genetic and environmental factors may also play a role, clinical and pre-clinical evidence suggest a role for sex hormones and menopause in the pathogenesis of the disease, although the underlying mechanisms are not understood. To address this, we developed a novel PD mouse model by injecting a hybrid serotype AAV vector overexpressing human wild type alpha-synuclein into the substantia nigra and combined it with the new model of accelerated ovarian failure. This model better reflects the human menopause compared to more traditional models like ovariectomy that only partially mimic the human process. In this paradigm, we found that AAV.SNCA injected perimenopausal females have increased nigral neuronal loss and progression of motor deficits compared to pre-menopausal females, and similarly to what observed in males. Altogether, our data shows that the combination of these 2 novel mouse models has the potential to decipher the contribution of sex hormones to PD pathogenesis and pathology, and to unravel specific molecular mechanisms underlying sex dimorphism in PD neurodegeneration.

Parallel Session 37 – Ventral striatal dopamine and circuit function in reward-driven behavior

Dopamine D2 receptor upregulation in cholinergic interneurons (CINs) of the nucleus accumbens: effects on CIN function and motivated behavior

Eduardo Gallo[1]
[1]*Fordham University*

BACKGROUND AND AIM: Cholinergic interneurons (CINs) of the NAc have emerged as key regulators of striatal function by modulating striatal plasticity and regulating local dopamine (DA) release. While CINs express DA D2 receptors (D2Rs), little is known regarding the consequences of DA signaling in modulating CIN function and mediating reward-related behaviors. In response to presentation of reward-related stimuli, CINs exhibit transient changes in firing patterns, including a DA-dependent "pause" which is thought to be important for associative learning. Ex vivo studies have also implicated D2Rs as mediators of the DA-dependent CIN pause. Yet, the specific role of D2Rs in shaping the pause response in NAc CINs and its involvement in motivated behavior remain unclear. To this end, we used a combination of slice electrophysiology, in vivo fiber photometry and behavioral analysis in mice. METHODS: To determine the role of CIN D2Rs in CIN function, we first examined whether virus-mediated overexpression of D2R alters CIN firing in response to DA. We prepared acute NAc slices from ChAT-Cre x DAT-Cre mice expressing either D2Rs or EGFP in NAc CINs and ChR2 in midbrain DA neurons.

Using cell-attached recordings, we measured CIN firing in response to 20-Hz optogenetic stimulation of DA terminals. Next, we sought to determine if CIN D2R upregulation would alter CIN function in vivo. We expressed a genetically encoded GPCR-Activation Based acetylcholine (ACh) sensor (GRAB) in NAc to measure ACh levels using fiber photometry during a continuous reinforcement schedule. Finally, to determine the behavioral impact of CIN D2R upregulation, we used a go/no-go task, a NAc and DA-dependent task that tests the ability to withhold a response depending on whether a go or a no-go stimulus is presented. RESULTS: In slice, NAc CINs overexpressing D2Rs showed a significant pause elongation compared to CINs expressing EGFP, which showed only a brief reduction in tonic firing (D2R: 2.18 ± 0.28 s, n = 22; EGFP: 0.77 ± 0.11 s; $p < 0.005$, n = 20), without altering the baseline firing. Blocking D2Rs with sulpiride abolished the pause in both conditions, indicating that D2Rs are necessary for the DA-induced pause in NAc CINs. In vivo, our initial fiber photometry data reveal distinct alterations in GRAB signal following lever presentation, typically featuring a transient rise followed by a reduction in signal in both trained EGFP and D2R-overexpressing mice. Early analyses suggest that the event-related dip in GRAB signal is more prominent and more sustained following CIN D2R upregulation. In the go/no-go task, D2R upregulation led to higher error rates during no-go trials, without affecting performance on go trials. CONCLUSIONS: These findings suggest that, by regulating the CIN pause evoked by phasic DA, NAc CIN D2Rs participate in appropriate cue-related responding for reward. Further, these results position CINs as an important target of NAc DA in motivated behavior.

Dopamine D2Rs coordinate cue-evoked changes in striatal acetylcholine levels

Christoph Kellendonk[1]
[1]Columbia University

In the striatum, acetylcholine (ACh) neuron activity is modulated co-incident with dopamine (DA) release in response to unpredicted rewards and reward predicting cues and both neuromodulators are thought to regulate each other. While this co-regulation has been studied using stimulation studies, the existence of this mutual regulation in vivo during natural behavior is still largely unexplored. One long-standing controversy has been whether striatal DA is responsible for the induction of the cholinergic pause or whether D2R modulate a pause that is induced by other mechanisms. Here, I will present data using genetically encoded sensors to simultaneously measure ACh and DA levels during behavior after genetic or pharmacological D2R inactivation. We found that the induction of cue induced decrease in ACh levels is not dependent on CIN D2Rs. Rather, D2Rs regulate the lengths of the decrease and inhibit ACh rebound levels. Notably, the change in task-evoked ACh levels is not associated with altered DA release. At the behavioral level the cue induced decrease in ACh levels is correlated with the latency to press and artificial inhibition of CINs revealed that longer inhibition shortens the press latency compared to shorter inhibition. This suggests a role of the cue-induced ACh signal in the motivation to initiate actions.

Role of nucleus accumbens D1- and D2-MSNs in rewarding and aversive behaviors

Ana Rodrigues[1]
[1]University of Minho

BACKGROUND AND AIM: The nucleus accumbens (NAc) is a core region modulating rewarding and aversive behaviors. The NAc is mainly composed of medium spiny neurons (MSNs), segregated into those expressing dopamine receptor D1, dynorphin and substance P (D1-MSNs) or dopamine receptor D2 and enkephalin (D2-MSNs). The classic model of striatal function proposes that D1-MSNs encode/drive reward whereas D2-MSNs encode/drive aversion. However, evidence from our team and others challenged this model of functional opposition, suggesting that this dichotomy may be overly simplistic. METHODS: We used optogenetics to specifically manipulate NAc D1- or D2-MSNs activity in different behavioral tests. In addition, we performed in vivo electrophysiology to characterize neuronal activity changes induced by MSN optogenetic manipulation in downstream regions, namely the ventral tegmental area (VTA) and ventral pallidum (VP). RESULTS: Brief D1- or D2-MSN optogenetic stimulation elicited positive reinforcement and enhanced cocaine conditioning. Conversely, prolonged activation of either population induced aversion, and in the case of D2-MSNs, decreased cocaine conditioning. Part of these behavioral effects were dynorphin- and enkephalin-dependent. In addition, brief and prolonged stimulation induced distinct electrophysiological effects in downstream target regions, namely the VTA and VP. CONCLUSIONS: Our findings demonstrate that D1- and D2-MSNs can bi-directionally control reward and aversion, explaining the existence of controversial studies in the field, and highlights that the proposed striatal functional opposition needs to be reconsidered.

Mechanism underlying drug abuse vulnerability driven by low levels of striatal dopamine D2 receptors

Veronica Alvarez[1]
[1]National Institutes of Health

Low levels of striatal dopamine D2 receptors has been shown in humans that abuse drug and also in individuals with traits that predispose to compulsive drug use. Research over the past two decades has solidified this link but the mechanism underlying this vulnerability remains unknown. Veronica Alvarez will present preclinical studies, a combination of published and unpublished data from mouse models, that offer evidence for a causal link between low D2 receptor in selective populations of neurons within the basal ganglia and the vulnerability to cocaine and alcohol abuse. The findings also provide a mechanistic understanding of how low levels of D2 receptors in selective cell-types contribute to different aspects of the vulnerability.

Parallel Session 38 – Role of neuromodulators in synaptic plasticity and memory

Role of Locus Coeruleus-Norepinephrine System in the Cerebellum during Fear Conditioning

Maria Miniaci[1], Adrien Stanley[2], David Sulzer[2]
[1]University of Naples Federico II, [2]Columbia University

Norepinephrine (NE) is a neuromodulator involved in a broad variety of brain processes, including attention, arousal, decision making, and memory. The cerebellar cortex receives a widespread noradrenergic projection from the locus coeruleus which is consistent with the evidence that the norepinephrine system is involved in the modulation of cerebellar functions including motor learning. This study aimed to determine whether the NE projections to the cerebellum are involved in the memory formation of emotionally arousing events. For this purpose, we examined how fear conditioning affects the release of NE in the cerebellum and the effect of inhibition of cerebellar NE

projections on fear response using chemogenic or optogenetic tools. We demonstrate that, following fear conditioning, the conditioned stimulus elicits the release of NE in the cerebellum. In addition, we show that the selective inhibition of cerebellar NE terminals reduces conditioned fear without affecting balance and motor coordination. According to our electrophysiological data, NE modulates one of the main excitatory synapses in the cerebellum, i.e. the parallel fiber- Purkinje cell (PF-PC) synapse, by acting on α- and β -ARs. In particular, the activation of α-ARs produces synaptic depression between PFs and PCs whereas β2-AR activation facilitates the PF-PC synaptic potentiation. We hypothesize that this double mechanism of regulation of PF-PC synaptic transmission by NE may serve to decrease the background activity of PCs and enhance the excitatory signals arriving at PCs via PF. This mechanism may allow the NE fibers to refine the signals arriving at Purkinje cells at particular arousal states or during learning.

Dopamine-dependent synaptic plasticity and alpha-synuclein: implication for motor and memory dysfunctions

Paolo Calabresi[1]
[1]Gemelli University Hospital

Over the last two decades, many experimental and clinical studies have provided solid evidence that alpha-synuclein (α-syn), a small, natively unfolded protein, is closely related to Parkinson's disease (PD) pathology. However, the role of this protein in dopamine (DA) dependent striatal synaptic transmission and plasticity is still unclear. In fact, while DA release machinery alterations have been considered a primary expected effect of α-syn toxicity, recent studies have focused on the postsynaptic counterpart of this pathological scenario. Altered activity and distribution of postsynaptic density components have only recently been explored but may be promising tools to detect subtle but measurable changes at the core of this synaptopathy. By utilizing electrophysiological, behavioral, and molecular analyses we have investigated the role of α-syn on long-term potentiation (LTP) and long-term depression (LTD), the two main forms of striatal synaptic plasticity. These forms of synaptic plasticity are both represented at corticostriatal synapses and strongly depend on the activation of DA receptors. We found that LTP and LTD in striatal spiny neurons and cholinergic interneurons are altered in a distinct manner in various models of synucleinopathies. Moreover, we also found that striatal plasticity is differentially affected in the early stages of the disease, in the absence of DA depletion, as well as in later stages involving DA denervation. Our findings suggest that different therapeutic approaches might be considered in the different phases of PD targeting both DA as well non-DA dependent mechanisms.

Altered metabolism of heparan sulfate leads to developmental dopaminergic abnormalities responsible for autistic-like behaviours in lysosomal storage disorders

Elvira De Leonibus[1]
[1]Institute of Neurobiology and Cellular Biology

Lysosomal storage disorders (LSDs) including Mucopolysaccharidosis (MPS) III are characterized by altered metabolism of heparan sulfate (HS) and exhibit lysosomal dysfunction leading to neurodegeneration and dementia in children, preceded by severe and therapy-resistant autism (ASD). Altered metabolism of HS has been found in both animal models and in patients with iatrogenic ASD,

however the mechanisms through which HS contributes to ASD are unknown. Since HS is a coreceptor of many growth factors that regulate the development and of the dopaminergic system, we examined whether the dopamine (DA) system is affected during neurodevelopment in LSDs-linked autism. Using different in vitro models, we identified an increased proliferation of dopaminergic progenitors; replacement of functional HS was sufficient to rescue the DA proliferative phenotype. Using a mouse model of MPS-III we confirmed an increased number of DA cells during adult life, associated to an imbalance between D1- and D2-like receptor pathways in the striatum. Finally, through a set of in vivo pharmacological experiments we showed that not only limiting DA synthesis, but also re-balancing D1-D2-like receptor activation by administering the D1-like receptor antagonist SCH-23390, rescued autistic-like behaviours in young adult MPS-IIIA mice. These findings indicate that embryonic dopaminergic neurodevelopmental defects due to altered function of HS may lead to autism in LSDs, but also to iatrogenic autism. Thus, we discovered that in LSDs lysosomal dysfunction leading to dementia runs in parallel to HS-neurodevelopmental pathology leading to autism, which needs disease' specific DA-based treatments. This study was supported by grants from Sanfilippo Children's Foundation, Cure Sanfilippo Foundation, Sanfilippo fighters Foundation to EDL

Regulation of presynaptic mitochondrial transport by serotonin

Sathya Puthanveettil[1], Kerriann Badal[1], Komol Akhmedov[1], Phillip Lamoureux[2], Xin-An Liu[1], Kyle Miller[1]
[1]The Scripps Research Institute, [2]Michigan State University

Despite the progress in our understanding of the mechanisms by which modulatory neurotransmitters mediate learning, whether and how these neurotransmitters modify the bidirectional communication between the cell body and synapse for regulating synaptic transmission and structural plasticity is poorly understood. To address this, by live imaging we have quantified the temporal changes in the bidirectional mitochondrial transport in the well-defined pre and post-synaptic neurons of Aplysia gill withdrawal reflex. We find that the flux of the bidirectional mitochondrial transport is enhanced specifically for the initiation of long-term facilitation (LTF) produced by the five spaced applications of the serotonin but not for the maintenance of LTF. We then assessed whether serotonin might also regulate transport of other organelles and found that serotonin specifically regulate retrograde transport of lysosomes during initiation. Taken together, these results establish that serotonin regulate the organelle transport in unique ways for facilitating the bidirectional communication between the cell body and the synapse during initiation of long-term memory storage.

Sexually dimorphic dopamine signaling dictates the penetrance and behavioral trajectory of human dopamine transporter coding variation

Adele Stewart[1]
[1]Florida Atlantic University

The dopamine transporter (DAT) Val559 variant, identified in patients with ADHD, bipolar disorder, and autism spectrum disorder, supports non-vesicular dopamine leak we termed anomalous dopamine efflux (ADE) and leads to homeostatic dopamine disruptions in mice harboring the mutation. We now

report profound, sex-dependent phenotypic divergence in DAT Val559 mice that derives, at least in part, from circuit-specific dopamine D2 receptor (D2AR)-DAT coupling. In male DAT Val559 mice, presynaptic nigrostriatal D2ARs are constitutively-activated, driving enhanced DAT surface trafficking and phosphorylation, an effect is absent in DAT Val559 females. However, females show constitutive D2AR activation of presynaptic mesolimbic D2ARs. In parallel we have identified region-dependent, sex-specific differences in DA clearance and sexually dimorphic behaviors both at baseline and in response to DAT-targeted drugs. Our work provides a cogent example of how a shared biological insult drives alternative physiological and behavioral trajectories due to a sex biased neuronal architecture.

Interactions between synaptic zinc, dopamine, and cocaine as a function of sex

Oscar Solis[1]
[1]National Institute on Drug Abuse Intramural Research Program

Glutamatergic signaling in the striatum is implicated in cocaine use disorder. Within a subset of glutamatergic neurons, vesicular zinc (Zn2+) is co-released with glutamate and modulates dopamine transmission. Previous studies demonstrated that estrogen reduces Zn2+ in the brain and that Zn2+ exerts sexually dimorphic effects on locomotion and at skilled motor learning tasks. Recently, we found that cocaine increases Zn2+ levels in the striatum, and that Zn2+ increases the in vivo potency of cocaine by binding to the dopamine transporter (DAT). In line with these findings, we showed that the absence of vesicular Zn2+ induces a decrease in conditioned place preference, locomotor sensitization and self-administration to cocaine. In this talk I will present data describing interactions between sex, Zn2+, and DAT in relation to cocaine exposure.

Sex-specific behavioral strategies to elucidate neurobiology of adaptive decision-making

J.E. Zachry[1], *M.G. Kutlu[1], E.S. Calipari[1]
[1]Vanderbilt University

In recent years, research focused on understanding the biological variables contributing to psychiatric disorders has highlighted sex-based differences in the development and presentation of symptoms as well as in fundamental behavioral processes. A large body of work has focused on understanding stimulus-driven behavior, sex differences in these processes, and the neural circuits underlying them. Sex-based differences in reward seeking and avoidance offer an ideal model to explore bias and strategy in a behavioral task while providing insight into the neurobiological basis of information encoding. For example, even though females will self-administer opiates at higher rates than males, when given a choice between opiates and a high-fat reward they choose the non-drug reinforcer over the drug alternative at a higher rate than males, clearly highlighting that sex differences do not manifest themselves as universal behavioral principles, but rather are a complex interaction between sex and environment. Further, work in rats has shown while females are more motivated to self-administer drug and non-drug rewards, they are also more sensitive to punishment. Numerous other studies have varied the magnitude, value, and probability of rewards highlighting that females are more risk averse than males. Capturing this complexity necessitates behavioral tasks that can probe the balance in the subjective value of rewarding versus aversive stimuli and their antecedent cues. We established a task—the Multidimensional Cue Outcome Action Task (MCOAT)—that allows for quantitative assessment of multidimensional behavioral functions relevant to human decision making in mice. By combining negative reinforcement, punishment, and positive reinforcement we can dissociate action from stimulus valence. First, we identified sex-specific behavioral strategies, showing that females prioritize avoidance

of negative outcomes over seeking positive, while males have the opposite strategy. Next, we showed that while males were more sensitive than females to unsignaled shocks of varying intensities, as measured by motor response and vocalizations, females are more sensitive to punishment. Our findings suggest male mice are more sensitive to lower shock intensities, while females are more sensitive to the effects of shock on reinforcement/punishment. Lastly, in order to understand how real-time activity in these populations is linked to behavioral execution, we expressed the genetically encoded calcium indicator (GCaMP6f) within D1 and D2 MSNs. We coupled this calcium indicator with in vivo fiber photometry and single-photon cellular resolution calcium imaging to record from these cell populations in awake and behaving animals to identify correlated changes in the calcium signal that relate to behavioral differences in male and female mice.

A population-wide g-protein coupled receptor atlas of spiny projection neurons identifies novel modulators of striatal activity

Mattias Rickhag[1]
[1]Copenhagen University Hospital Amager and Hvidovre

In Parkinson's disease (PD), progressive loss of dopaminergic innervation to striatum causes an imbalance in activity of striatal projection neurons (SPNs); dSPNs become hypoactive while iSPNs become hyperactive resulting in motor deficits manifested as tremor, bradykinesia and rigidity. While the dopamine receptors, which belong to the superfamily of G protein-coupled receptors (GPCRs), have been the foremost studied biological target in PD, other GPCRs expressed in SPNs are much less characterized. We envision that yet poorly characterized striatal GPCRs, other than the dopamine D1-receptor and D2-receptor, constitute a tractable approach to restore a balanced dSPN/iSPN activity in PD. For this purpose, we have generated a cell population-wide GPCR expression atlas from SPNs by combining fluorescence-activated cell sorting using D1R-TdTomato and D2R-GFP reporter mouse lines followed by extensive quantitative PCR (qPCR) arrays. Custom-made qPCR array analysis revealed several novel GPCRs with preferential expression in either dSPNs or iSPNs. Target GPCR activation in SPNs were studied at single-neuron level by expression of genetically-encoded biosensors (calcium/protein kinase A sensor) in order to determine their modulation of SPN activity. We characterize unexplored GPCRs with strong effect to modulate activity of SPNs and demonstrate the significance of metabotropic input to SPNs. Overall, we present a comprehensive depiction of the GPCR repertoire in SPNs and identify novel modulators of striatal signaling with a therapeutic potential in PD.

Dynamics of dopamine signal integration in striatal neurons

Pierre Vincent[1], Ségolène Bompierre[1], Cédric Yapo[1], Anu Nair[2], Elia Mota[1], Jeanette Kotaleski[3], Liliana Castro[1]
[1]Sorbonne Université - CNRS, [2]KTH Royal Institute of Technology, [3]Karolinska Institutet

Striatal Medium-sized Spiny Neurons (MSNs) integrate dopamine signals through the cAMP-PKA signaling pathway. Although the signaling enzymes involved in this integration are well identified, their respective contributions to the dynamics of signal processing remain unclear. We used biosensor imaging in mouse brain slice preparations to analyze the cAMP and PKA signals triggered by transient dopamine stimulations. In silico simulations were used to test SPN's responsiveness to various dynamic

dopamine signals. D1 and D2 receptors, expressed by two separate sub-classes of MSNs, showed a similar sensitivity to dopamine. The D1 response was efficiently suppressed by cholinergic agonists activating M4 muscarinic receptors, while the D2 receptor suppressed adenosine A2A signals. PDE10A appeared as the only PDE able to decrease cAMP concentration below micromolar level, and its activity was therefore required to deactivate PKA. PDE1B was shown to mediate glutamate - dopamine interactions, while PDE2A mediated a cross-talk between nitric oxide (NO) and dopamine. PDE2A and PDE4 appeared as modulators of peak dopamine responses. PKA-dependent phosphorylation appeared highly non-linear, probably as a result of DARPP-32-mediated inhibition of phosphatases. Overall, our data show that D1 MSNs are geared to respond in an all or none way to transient increases in dopamine. In contrast, D2 MSNs respond to transient lack of dopamine. Such dynamic description of signaling integration is required to better understand the effects of novel drugs, and define novel therapeutic strategies for diseases affecting the dopaminergic system.

Dopaminergic reward and performance prediction error signal are gated during courtship

Andrea Roeser[1], Vikram Gadagkar[1], Pavel Puzerey[1], Brian Kardon[1], Anindita Das[1], Jesse Goldberg[1]
[1]Columbia University

How do social interactions affect dopaminergic (DA) responses to rewards and performance outcomes? We used electrophysiology and fiber photometry to record DA signals in two mesostriatal pathways as thirsty male songbirds sang alone and to females. When alone, singing-related performance error signals were restricted to a song-specialized mesostriatal pathway; reward prediction error signals were observed globally. When singing to a female, DA responses to both water reward and song performance outcomes were diminished and were instead driven specifically by female calls that interrupted the song. Together, we discover that reward and performance error signals are differentially routed through distinct DA pathways, that DA signals dynamically change their tuning during courtship, and that an affiliative social interaction, when precisely timed, activates distinct DA systems.

L-type channel control of DA release is gated by endogenous regulators, can we utilise them as neuroprotective strategies against Parkinson's disease?

Katherine Brimblecombe[1], Stephanie Cragg[1]
[1]University of Oxford

SNc and VTA dopamine (DA) neurons, which project to dorsal and ventral striatum respectively differ in a number of ways. Notably in their high and low sensitivity to parkinsonian degeneration respectively. We have previously identified that DA release is differentially gated by L-type voltage-gated Ca2+ channels (LTCC) in the dorsal and ventral striatum. Given that LTCC function has been identified as a stressor of DA neurons at risk for parkinsonian degeneration we are interested in identifying the molecular mechanisms regulating LTCC function. Using fast-scan cyclic voltammetry in acute ex-vivomouse brain to access mechanisms regulating LTCC control of DA release across striatal territories in both sexes. We identify calbindin-D28K as limiting LTCC function in a regionally and sexually divergent manner: D2-receptors and DA-transporters as negative and positive regulators of LTCC respectively and lastly find that targeting α2δ subunits with gabapentinoid drugs limits LTCC function without compromising DA release. Therefore LTCC-function can be dynamically and locally regulated which may prove critical for future neuroprotective strategies.

Altered intrinsic connectivity within striatal subregions is associated with anhedonia as a function of striatal tissue iron levels among youth with depression

Cecile Ladouceur[1], Teague Henry[2], Amar Ojha[1], Rasim Diler[1]
[1]University of Pittsburgh, [2]University of Virginia

Anhedonia–a core symptom of depression that leads to poor outcomes–is associated with alterations in the dopaminergic reward system, including the striatum. This study examined, among adolescents varying in levels of depression, whether resting state striatal regional homogeneity (ReHo)–an index of intrinsic regional connectivity shown to be reduced in adult depression–was associated with symptoms of anhedonia and to what extent this relationship may be moderated by striatal tissue iron, an index of dopamine(DA) function. Participants (12-17 yrs old, n=75) varying in depression symptoms completed clinical assessments and a resting state fMRI session. ReHo and mean standardized T2* (inverse proxy of tissue iron, itself a proxy for DA concentration) were calculated within the striatum. To examine the relationships between ReHo, mean T2* intensity, and anhedonia symptoms, we used a voxel-wise moderated mediation approach. Results showed that reduced ReHo was associated with higher levels of anhedonia with higher levels of tissue iron concentration in the right caudate (peak T=4.17), and with lower levels of anhedonia in adolescents with lower levels of tissue iron concentration in the same region (peak T=2.99). Lower tissue iron concentration in the left putamen was associated with higher levels of anhedonia overall (peak T=2.79). Findings indicate that intrinsic connectivity in subregions of the striatum is associated with anhedonia but the direction of this relationship is contingent upon striatal dopaminergic function. Such findings point to the need to examine whether dopamine-targeted pharmacotherapy may be effective for a subset of adolescents with anhedonia.

Poster Abstracts

Poster Session 1

A – Dopamine, motivation, reward and addiction

1-A-1 Leveraging CRISPR/Cas9 gene editing technologies to determine the regulators of dopamine physiology and behavior

Barbara Juarez[1]
[1]University of Washington- Seattle

The midbrain dopamine system is comprised of dopamine neurons originating from the ventral tegmental area projecting to downstream neural substrates that are involved in a number of behavioral domains such as reward, learning and executive function. These neurons exhibit highly coordinated tonic and phasic activity patterns and it is increasingly believed that loss of precise dopamine underlies a number of neurological and neuropsychiatric disorders. Understanding the regulators of tonic-phasic balance could lead to new insight into the underlying basis of healthy and pathological behaviors. Voltage-gated potassium channels are important regulators of neural excitability and firing. Here, we sought to elucidate how two voltage-gated potassium channel subunits thought to regulate tonic (Kv4.3) and phasic firing (KCa1.1) exert their regulatory action on the midbrain dopamine activity to modulate distinct behavioral domains. We targeted viral-based CRISPR/Cas9 mutagenesis to the coding regions of two potassium channel subunits: Kv4.3 (Kcnd3) and KCa1.1(Kcnma1) of midbrain dopamine neurons in adult DATiCre mice. Using patch clamp electrophysiology, we found that mutagenesis of Kv4.3 and KCa1.1 impart distinct neurophysiological characteristics on midbrain dopamine neurons of adult mice. We also found that these two potassium channels regulate specific behavioral domains known to be regulated by midbrain dopamine neurons such as locomotion, anxiety and social behaviors. We also observed distinct effects on reward-associated behaviors between these two channel knockouts. These models have elucidated how dopamine neuron tonic-phasic balance contribute to healthy behavioral function in mice.

1-A-2 The effects of continuous, low dose amphetamine treatment on behavioural features of cocaine addiction in female rats

Ndeye Aissatou Ndiaye[1], Florence Allain[2], Anne-Noël Samaha[2]
[1]Samaha Lab, [2]Université de Montréal

There are no approved medications to treat cocaine addiction. Continuous, low-dose d-amphetamine treatment is considered the most promising pharmacological strategy to reduce cocaine use. In both male rats and human cocaine users, low-dose amphetamine treatment reduces cocaine taking and seeking. However, there are no published studies on this issue in female animals, even though in humans, cocaine addiction afflicts both women and men, and women can be more vulnerable to the disorder. Here, we will assess in female rats the effects of continuous, low-dose amphetamine treatment (AMPH) on the development and expression of addiction-relevant patterns of cocaine use. Female rats will first receive 14 cocaine self-administration sessions (1/day). We will then establish baseline levels of responding for cocaine under a progressive ratio schedule (measure of motivation to take cocaine). The rats will then be allocated to 2 groups, and allowed to self-administer cocaine daily for 2 weeks. One group will receive AMPH (5 mg/kg/day, via minipump) during the 2 weeks. The other group will not receive AMPH. Finally, after the cessation of cocaine self-administration and AMPH treatment, we will re-assess the motivation to take cocaine as well as cocaine-induced relapse

behaviour. If AMPH effects in this context are similar in female and male rats, then AMPH should decrease both the motivation to take cocaine and relapse-like behaviour after abstinence.

1-A-3 Dissociable activity dynamics in projection-specific midbrain dopamine subpopulations contribute to reward association and motivation

Jordan Elum[1], Scott Ng-Evans[1], Grigory Loginov[1], Larry Zweifel[1]
[1]University of Washington

The mesolimbic dopamine (DA) system regulates both reinforcement learning and motivation. A central question is how the mesolimbic DA system influences these distinct functions. A classical neural circuit model proposes a two-component system in which DA release in the nucleus NAc core encodes prediction error signals to regulate reinforcement learning while DA release in the nucleus NAc shell signals incentive salience and promotes motivated responses. Here we record bulk calcium dynamics in projection-specific ventral tegmental area (VTA) DA populations during a cued reinstatement task. We show that NAc core- and shell-projecting DA populations are activated by actions, cues, and rewards. However, we find differential activity dynamics in action, cue, and reward encoding between projection-specific DA populations. NAc shell-projecting VTA DA neurons preferentially encode animals' action initiation (lever press) and display a sustained increase in activity during the cue and reward outcome periods. In contrast, these signals in the NAc core-projecting population return to baseline between discrete events. During unexpected reward omission, the NAc core-projecting population displays temporally discrete decreases in calcium signals consistent with a prediction error-encoding model that are not observed in the shell-projecting DA cells. By optogenetically manipulating both the NAc shell-projecting and core-projecting VTA DA populations we find that these populations differentially regulate motivation during cued reinstatement. These findings suggest that NAc core-projecting DA neurons provide prediction error signals to facilitate reinforcement learning while NAc shell-projecting DA neurons provide incentive salience signals to promote motivated behavioral responses.

1-A-4 Methamphetamine and fentanyl co- self-administration modifies fentanyl taking and exacerbates dopamine deficits in the nucleus accumbens

Monica Dawes[1], Katherine Holleran[1], Sara Jones[1]
[1]Wake Forest School of Medicine

Due to the recent increases in concurrent methamphetamine and fentanyl use and fentanyl-associated overdose deaths, it is vital to examine the interactions between these two substances. Male and female Long Evans rats were trained to self-administer 2.5 µg/kg/inf fentanyl. Following acquisition (2 consecutive days of 20 infusions), rats were randomly assigned to either fentanyl alone or fentanyl methamphetamine and were tested on a short access, fixed ratio 1 schedule of reinforcement (3 hr sessions, max. 20 infusions) for ascending doses of fentanyl or combined fentanyl methamphetamine (1.25, 2.5, 5.0 µg/kg/inf fentanyl ± 0.1 mg/kg/inf methamphetamine, 5 days per dose). Following self-administration, coronal brain slices containing the nucleus accumbens were prepared for ex vivo fast scan cyclic voltammetry. Rats self-administering the combination of fentanyl and methamphetamine completed their sessions more quickly, and had shorter latency to initiate responding than animals administering fentanyl alone at moderate and high doses of fentanyl. Combined fentanyl and methamphetamine rats had decreased evoked dopamine release and uptake rate (Vmax) compared to saline and fentanyl alone animals. Further, evoked dopamine release was decreased across stimulation amplitudes (1.4-10V) and in response to stimulation trains (5 pulses; 5, 10, 20, and 100Hz) in combined

fentanyl and methamphetamine animals, compared to fentanyl alone animals. Together, these results highlight the complexities of combined opioid and stimulant use, and suggest that there may be unique neuroadaptive processes specific to combined fentanyl and methamphetamine which are not sufficiently explained by the individual changes observed following use of fentanyl or methamphetamine alone.

1-A-6 High saturated fat diet interacts with a D2 receptor expression based polygenic risk score to predict reversal learning

Gibson Weydmann[1], Euclides José de Mendonça Filho[1], Barbara Barth[2], Sachin Patel[1], Irina Pokhvisneva[1], Robert Levitan[3], Michael Meaney[1], Lisiane Bizarro[4], Patricia Silveira[1]
[1]Douglas Mental Health University Institute, McGill University, [2]McGill University, [3]University of Toronto, [4]Universidade Federal do Rio Grande do Sul (UFRGS)

Obesity is associated with impairments in reversal learning. These impairments might reflect the effect of fat consumption over D2 receptors availability and binding on the striatum (STR) and ventral tegmental area (VTA). However, diet effects may be moderated by early genetic differences. In this study, we tested how two novel expression-based polygenic scores, constructed based on the co-expression gene network of the DRD2 gene on the striatum and ventral tegmental area (ePRS-STR/ePRS-VTA) moderate the effect of saturated fat consumption over learning. Generalized estimation equation models (GEE) were applied using outcomes from the Intra-Extra Dimensional Set Shift (IED) task that assessed learning by reward and reversal learning at 48, 60, and 72 months of age in the MAVAN cohort. Sex, population stratification, gestational age, zBMI, and sugar consumption were used as covariates. Interactions between saturated fat consumption, ePRS (ePRS-STR or ePRS-VTA) and learning over time were analyzed. Results revealed a significant two-way interaction between fat consumption and ePRS-STR (B = − 5.927, p = .010), in which the number of errors in reversal learning increases as saturated fat consumption increases for those children with lower (- 1 SD) ePRS-STR score. A significant three-way interaction suggested that these interactions weakened with age (B =.076, p = .032). None of the models predicted learning by reward. Also, ePRS-VTA interactions were non-significant. To our knowledge, this is the first study in humans showing that saturated fat consumption impacts reversal learning. The use of a novel genomic approach permitted the finding that diet impact over learning might be moderated specifically by the action of genes co-expressed with the striatal DRD2 gene.

1-A-7 Recreational but not therapeutic-like amphetamine exposure in adolescence disrupts mesolimbic dopamine dynamics in adulthood

Janet Zhao[1], Giovanni Hernandez[1], Del MacGowan[1], Tanya Capolicchio[1], Aoran Song[1], Areesha Moiz[1], Cecilia Flores[1]
[1]McGill University

The mesocorticolimbic dopamine (DA) system exhibits an extended development throughout adolescence. This protracted development makes it malleable to external factors, including drugs of abuse. Indeed, adolescent mice exposed to a recreational- but not a therapeutic-like dose of amphetamine (4 and 0.5 mg/kg, respectively) show sexually dimorphic alterations in the mesocorticolimbic DA connectivity in adulthood. Here, we investigated whether these alterations were associated with changes in DA release. Male and female mice received i.p. saline or amphetamine (0.5 or 4mg/kg) injections from PND22 to PND31. Drug rewarding effects were assessed using the conditioned place preference paradigm, with preferences tests conducted at PND32 and PND80. One

day after the adult preference test, mice were microinfused with a fluorescent DA sensor into the nucleus accumbens. Mesolimbic DA dynamics were measured with fiber photometry in response to i.p. administration of (i) saline and (ii) methylphenidate (MPH, 10mg/kg). In males, recreational- but not the therapeutic-like dose of amphetamine in adolescence led to short and long-lasting place preference and exaggerated nucleus accumbens DA release in response to MPH, compared to saline pretreatment. In contrast, in females, both recreational- and therapeutic-like amphetamine in adolescence induced short and long-lasting place preference, but only the recreational dose led to heightened MPH-induced mesolimbic DA release. MPH-induced DA release was higher in females than in males across groups. These results show that females are more sensitive to the enduring rewarding effect of amphetamine in adolescence than males, but, in both sexes, only recreational-like exposure sensitizes adult mesolimbic dopamine release.

1-A-8 Role of dopamine neurons in inter-individual variability during social labor division task in mice

Clément Solié[1], Robin Justo[1], Sophie Fayad[1], Fabio Marti[1], Tinaïg Le Borgne[1], Philippe Faure[1]
[1]CNRS - ESPCI - PSL Research University

Individuals living in group have to adapt to the physical and social constraints of the environment. In that context, access to shared resources leads to the emergence of specific strategies, such as competition or cooperation and division of labor among individuals. To assess individual adaptation and inter-individual variability, we developed a task where mice are tracked continuously across several days in an environment where the food is accessible through a lever press. The lever and the food magazine are far apart and reachable by all the mice at any moment. When living in triads, different individual strategies emerge for food access. Interestingly, when mice are performing the task alone, different behaviors also appear illustrating that a same goal can be achieved in different manners, independently of social pressure. We aim at understanding the neuronal basis of these individual variations. Dopamine (DA) neurons of the ventral tegmental area (VTA) are known to be involved in social behaviors and decision making. We therefore hypothesized that VTA DA neurons play a major role in the different strategies observed in the social labor division task. We address this question using in-vivo electrophysiology in anesthetized mice and fiber photometry technique in freely behaving animals to record VTA DA activity in the different animals' profiles.

1-A-9 Synaptic controllability of dopamine as a driver of adaptive behaviors

Sasha Burwell[1], Hai Dun Yan[1], Michael Tadross[1]
[1]Duke University

Ventral tegmental area dopamine neurons (VTA DA) fire in a manner consistent with Reward Prediction Error, with unexpected reward causing a burst in firing and unexpected reward omission producing a pause. Regarding pauses, optogenetic experiments have found that transient VTA DA silencing can drive behavioral extinction. However, silencing all dopamine neurons differs from the sparse nature of endogenous pauses, raising the question: which synaptic inputs to dopamine neurons contribute to natural pauses, and are they important drivers of behavior? We explore this question with an emerging technology, DART (drugs acutely restricted by tethering), which delivers clinical drugs to genetically defined cells. We combine this approach with a custom multiplexed behavioral task designed to assess conditioning and extinction simultaneously within-mouse. Our key finding is that acute GABA-A receptor antagonism at VTA DA neurons with gabazine-DART accelerates extinction with no impact on

conditioning. This effect confirms that GABA afferents are critical for extinction, but is opposite the canonical prediction that attenuating VTA DA pauses should slow the rate of extinction learning. We propose a model in which GABA inputs to dopamine neurons instead reflect expertise, encoding a strong prior achieved through extensive cue-reward association, with removal of this input thus weakening the prediction and enhancing extinction. This work provides critical insight into the circuitry underlying adaptive behaviors.

1-A-10 Detailed Characterization of the Effects of the Vesicular Monoamine Transporter-2 Inhibitor Tetrabenazine on Effort-based Decision Making and Binge-like Eating: Exertion of Effort vs. Anhedonia

Gayle Edelstein[1], Alev Ecevitoglu[1], Carla Carratala-Ros[1], Renee Rotolo[1], Rose Presby[1], Reighleigh Fleeher[1], Jamie Masthay[1], Merce Correa[2], John Salamone[1]
[1]University of Connecticut, [2]Universitat Jaume I

Brain dopamine (DA) transmission regulates exertion of physical effort and effort-based choice. Tetrabenazine (TBZ), a VMAT-2 inhibitor that blocks vesicular storage and depletes DA, alters effort-based choice by inducing a low-effort bias. TBZ induces depressive symptoms and motivational impairments in humans, and is used in rodents to model motivational dysfunctions. TBZ produces a low-effort bias in rats tested for effort-based choice using the concurrent fixed ratio (FR) 5/chow feeding choice task. At baseline, rats show a preference for FR5 lever pressing for the more palatable food (high-carbohydrate pellets) and eat little of the concurrently available lab chow. Detailed analyses of the temporal characteristics of lever pressing show that TBZ-treated rats (1.0 mg/kg IP) shift away from lever pressing, with a reduction in the number of completed ratios, a slowing of local rate within ratios, and an increase in total time spent in pauses from lever pressing. TBZ-treated rats showed a significant and substantial increase in intake of the concurrently available chow, increasing total grams consumed, total time spent eating, and number of bouts of chow feeding. Detailed temporal analyses showed that TBZ did not alter the total combined time spent lever pressing for pellets and consuming chow, but instead shifted the allocation of time away from lever pressing and towards chow intake, leaving fundamental aspects of food reinforcement intact. Separate studies of binge-like eating of chocolate in non-food-restricted rats showed that TBZ did not reduce chocolate intake, indicating that TBZ did not induce "anhedonia". These results have implications for modeling the motivational impairments seen in psychiatric disorders such as depression and schizophrenia.

1-A-11 Mu opioid receptor expression on dopaminergic axons of nucleus accumbens correlates with voluntary wheel running

Ikponmwosa Pat-Osagie[1], Chiye Aoki[1]
[1]New York University

Exercise is widely accepted to be beneficial for health and cognition, but it is challenging to convert sedentary adults to become regular exercisers. Why might this be and what might be the cellular substrates generating individual differences in voluntary exercise? We tested the hypothesis that individual differences in exercise, measured as voluntary wheel running of mice, may be attributed to individual differences in the expression of μ opioid receptors (MOR) at excitatory or inhibitory synapses or on dopaminergic axons in nucleus accumbens (NAcb), a brain region richly innervated by dopaminergic axons, heavily endowed with MOR and known to play a central role in encoding appetitive stimuli. Animals (2 females, 2 males) were singly housed starting mid-adolescence and each animal's

wheel running activity was monitored continuously (Med Associates, ENV-044) for 24 hours over 13 days. Dual electron microscopy was employed to quantify the extent of MOR expression on dopaminergic axons and at excitatory and inhibitory synapses of NAcb of four adult mice with widely varying (5-fold difference) running behavior. Brain tissue was preserved by transcardial perfusion with 4% paraformaldehyde and labeled for dopaminergic axons using anti-tyrosine hydroxylase and HRP-DAB as the electron-dense label and for MOR using anti-MOR and silver-intensified gold as the immunolabel. The results show a strongest trend for MOR on axons (R=-0.81, p=0.18, N=4), indicating that those animals with heightened MOR dopaminergic axons ran less. Albeit still only a trend, this finding suggests that endorphins, released during heightened exercise and activating MOR may reduce excitability of dopaminergic axons, thereby reducing the strength of appetitive stimuli and blunt wheel running.

1-A-12 Neurotransmission at D1 dopamine receptors underlies an aversive component of dopamine neuron self-stimulation

Milan Valyear[1], Noemie Eustachon[1], Irina Alymova[1], Jonathan Britt[1]
[1]McGill University

Dopaminergic inputs to the ventral striatum support self-stimulation behaviour and paradoxically these same inputs, at times, signal aversion and produce avoidant behaviours. To investigate the interplay between these rewarding and aversive components, we developed a novel self-stimulation procedure in which mice are trained to hold-down a lever for continuous self-stimulation. When we used lever hold-downs to trigger photostimulation of dopamine fibers in the ventral striatum, the mean duration of self-stimulation was about 3 seconds. This behaviour varied according to the within-bout frequency of stimulation. When we altered the stimulation frequency across 30 second intervals, we saw more rapid disengagement from higher frequency stimulations, even though they were more reinforcing. Cumulative time spent holding-down the active lever was highest for 40 Hz and lowest for 2.5 Hz trials, while mean hold-down duration was shortest for 40 Hz trials and longest for 2.5 Hz trials. The divergence of these measures suggests that there may be an aversive component to high frequency DA fiber stimulation. Pharmacological modulation of neurotransmission at D1 but not D2 receptors eliminated the divergence between cumulative and mean hold-down durations. Collectively, these results suggest that high frequency stimulation of ventral striatal dopamine inputs acquires aversive properties through a D1-receptor mediated process which mice mitigate by rapidly terminating stimulation trains.

1-A-13 Diametric changes in ventral striatal dopamine release underlie drug-taking and drug-seeking behaviors

Lauren Burgeno[1], Ryan Farero[2], Nicole Murray[2], Jenny Steger[2], Marta Soden[2], Larry Zweifel[2], Paul Phillips[2]
[1]University of Oxford, [2]University of Washington

Ventral striatal dopamine has been shown to both mediate drug satiety, and promote craving and drug-seeking. How might dopamine transmission in the same region signal both satiety and craving? Drug cues serve different purposes in different contexts. During drug-taking, cues confirm the success of drug-seeking actions and indicate imminent drug delivery, thus suppressing further drug-seeking. In contrast, during reinstatement paradigms, the same cues are presented unexpectedly during periods of abstinence, signaling possible drug availability nearby and promote drug-seeking. For nucleus accumbens core dopamine to both decrease drug-taking and increase drug-seeking, we hypothesized

that there must be a divergence in the dopamine signals evoked by drug-cues when they are presented in drug-taking vs. -seeking contexts. We used fast-scan cyclic voltammetry to measure drug-cue elicited dopamine responses over progressive drug use in both of these contexts in rats. We found that while cue-elicited dopamine transmission decreases over the course of weeks of drug-taking, dopamine responses to the same cues presented in a drug-seeking context increase. In addition, cue-elicited dopamine signals observed during drug-seeking increased further during prolonged abstinence in parallel with enhanced drug-seeking. To confirm these changes in dopamine mediate the observed changes in behavior, we optogenetically stimulated dopamine release to test the prediction that increasing cue-elicited dopamine release in drug-taking vs. -seeking contexts, would suppress drug-intake but promote drug-seeking. Our results demonstrate that these diametric changes in dopamine release not only coexist, but work together to mediate different but equally important, core symptoms of substance use disorders.

1-A-14 Distinct Roles of Cortical Catecholamines in Regulating Motivated Behavior and Striatal Dopamine

Mayank Kumar[1], Sharonda Harris[1], Jena Delaney[1], Carson Chavez[1], Alex Orr[1], Aditi kumari[1], Nikhil Urs[1]
[1]UNIVERSITY OF FLORIDA

Catecholamine neuromodulators dopamine and norepinephrine are implicated in locomotion, motivation, and cognitive behaviors. Although striatal dopamine signaling and circuitry are well established, the role of cortical catecholamines in regulating striatal dopamine dynamics and behavior is not clear. Microdialysis studies have shown that the norepinephrine transporter (NET) knockout mice are a unique model as they have elevated cortical catecholamines but reduced striatal dopamine levels. We asked the question whether altered catecholamine levels in the NET KO mice affect motivated behavior and striatal dopamine dynamics. We used a probabilistic reversal learning (PRL) task and a devaluation variable interval reinforcement (VI30/60) task to test effects of NET KO on reinforcement learning and goal directed behavior. The NET KO mice show enhanced reversals per session in the PRL task and were sensitive to devaluation in the VI task compared to WT littermates. Lesion of cortical norepinephrine did not change reversal learning but reduced average nosepokes per session and increased number of omissions, suggesting that cortical norepinephrine might predominantly regulate arousal and alertness, whereas cortical dopamine might regulate reinforcement learning via regulation of striatal dopamine. Using fiber photometry and dLight 1.2 we observed that in the PRL task, WT mice show positive striatal dopamine transients during WIN trials (reward received) but negative transients during LOSS trials (no reward). Our next steps are to test effects of cortical dopamine lesion on reversal learning and measure striatal dopamine transients in the NET KO mice. Our studies will identify previously unappreciated roles for cortical catecholamines in the regulation of striatal dopamine dynamics and motivated behavior.

1-A-15 The cerebellar value signaling to the dopaminergic centers

Junichi Yoshida[1], Leila Khatami[1], Jorge Vera[1], Maritza Onate[1], Julian Guarque-Chabrera[1], Victoria Lovallo[1], Yoshikazu Isomura[2], Farzan Nadim[3], Kamran Khodakhah [1]
[1]Albert Einstein College of Medicine, [2]Tokyo Medical and Dental University, [3]New Jersey Institute of Technology

Dopamine release from the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) is essential for movement initiation and reward-based reinforcement learning. Recent studies indicate

that the cerebellum, traditionally thought to contribute to fine motor control, also represents reward-related information. Our lab has previously shown that the cerebellum sends monosynaptic excitatory projections to both SNc (Cb-SNc) and VTA (Cb-VTA). We examined whether (1) these projections convey reward-related information tol dopaminergic neurons and, (2) the SNc and VTA receive different information from the cerebellum. We did this by monitoring bulk calcium signals in Cb-SNc and Cb-VTA simultaneously using fiber photometry in head-fixed mice during a Pavlovian task where mice were given a cue and delayed liquid reward. Both Cb-SNc and Cb-VTA activities were increased at the cue onset, and both showed a large reward-dependent increase whose magnitude was larger for sweet vs. regular water. However, while Cb-VTA had almost equivalent activation to both the cue and the reward, Cb-SNc showed much larger activation in response to the reward than the cue. Additionally, Cb-SNc activity was sustained until the mouse stopped licking, whereas Cb-VTA remained active even after the termination of licking. These findings indicate that the cerebellum sends reward value information to both SNc and VTA. However, considering the different putative roles of these two dopaminergic centers in processing movement and reinforcement, we expect that the cerebellar inputs to these two centers may have somewhat distinct consequences for behavior.

C – Dopamine, cognition and schizophrenia

1-C-16 Elevation of complement pathway-related transcripts in midbrain in schizophrenia cases who display cytokine-related high inflammation profiles

Cyndi Shannon Weickert[1], Debora Rothmond[2], Kate Robinson[1], Tertia Purves-Tyson[1]
[1]NeuRA, [2]Schizophrenia Research Laboratory NeuRa Foundation

Introduction: In people with schizophrenia, pro-inflammatory cytokine transcripts are elevated in midbrain. We hypothesised that complement initiator C1qA, effectors C3 and C4, and regulators decay-accelerating factor (DAF/CD55) and MAC-IP (membrane attack complex-inhibitory protein /CD59), mRNAs will be elevated in the midbrain in schizophrenia. Methods: C1qA, C3, C4, DAF and MAC-IP mRNAs were examined by qRT-PCR in 28 schizophrenia cases and 29 healthy controls. Three diagnosis/inflammatory subgroups were previously defined using cluster analysis of pro-inflammatory transcripts. T-tests or ANCOVA, were used to test for differences between inflammation/diagnosis subgroups. Spearmans correlations were used to determine relationships between illness duration, antipsychotics, gene expression. Results: C1qA, C3, C4 and MAC-IP mRNAs were increased (37%-107%) in the schizophrenia/high inflammation subgroup compared to controls ($F>5.51$, $p<0.05$), while DAF mRNA was unchanged ($p>0.05$). C1qA, C3 and C4 mRNA positively correlated with daily CPZ equivalents ($Rho>0.44$, $p<0.05$). Complement gene expression was strongly and positively correlated with markers of microglia (AIF), astrocytes (GFAP) and macrophages (CD163) in schizophrenia cases. Conclusions: Increased complement cascade synthesis in schizophrenia cases may contribute to midbrain pathophysiology. Increased complement transcripts correlate with glia, including microglia, astrocytes and macrophages. Increased MAC-IP supports that resident neurons or cells in midbrain may be attempting to protect themselves against increased complement activity. Increased antipsychotics in those patients with higher inflammation and higher complement could also indicate a more symptomatic patient requiring higher treatment doses.

1-C-17 Sex differences in reward-guided decision making in an autism-associated gene variant in mice

Madison Merfeld[1], Cathy Chen[1], Erin Giglio[1], Nicola Grissom[1]
[1]University of Minnesota

Gene variants associated with neurodevelopmental disorders, including copy number variation at the 16p11.2 region, are known to impact reward learning and corticostriatal function in mouse models. These impacts, however, appear to differ across sexes, raising the question of how sex modulates the influence of autosomal gene variants on reward processing and striatal dopamine neurobiology. We have previously observed robust differences in reinforcement learning strategies across males and females in a reward-guided restless bandit task, with male animals spending longer exploring options. We are using this task to understand how sex modulates the impact of 16p11.2 hemideletion on reward-guided cognition. Training data prior to bandit performance suggests that 16p11.2 hemideletion female animals complete an increased number of trials compared to sex-matched control animals. In the bandit task, we will examine the balance of explore versus exploit trials, and we hypothesize that we will see fewer exploratory trials in females compared to males, and in 16p11.2 hemideletion compared to wildtype. As the nucleus accumbens is critical in reward-guided learning such as bandit tasks, we will examine dopamine release in the nucleus accumbens core (NAcc) using dLight fiber photometry to determine whether dopaminergic signaling differs across sexes and between genotypes, and how that may contribute to differences seen in task performance. We hypothesize that 16p11.2 hemideletion animals will have increased dopamine release in the NAcc during rewarded trials, and this difference will be more pronounced in females.

1-C-18 Larger amphetamine-induced dopamine release is associated with better clinical outcome in patients with first-episode psychosis

Nicole Praschak-Rieder[1], Ana Weidenauer[1], Ulrich Sauerzopf[1], Martin Bauer[1], Lucie Batova[1], Irene Graf[1], Verena Pichler[1], Lukas Nics[1], Rupert Lanzenberger[1], Markus Mitterhauser[1], Siegfried Kasper[1], Harald Sitte[1], Wolfgang Wadsak[1], Matthäus Willeit[1]
[1]Medical University of Vienna

Antipsychotics exert their beneficial effects by reducing signal transmission at dopamine D2/3 receptors (D2/3R). Imaging studies have shown that baseline occupancy of D2/3R by dopamine as well as the uptake and storage capacity of the radiolabeled dopamine precursor [18F]FDOPA are directly related to antipsychotic treatment response and clinical outcomes in patients with schizophrenia and first-episode psychosis (FEP). Amphetamine induces massive release of dopamine into the extracellular space. Reductions in binding of D2/3R radioligands after amphetamine provide a semi-quantitative measure for dopamine release in the living human brain. Here we studied the relationship between the magnitude of amphetamine-induced dopamine release and one year clinical outcomes in drug-naïve patients with FEP. Patients underwent two positron-emission tomography (PET) scans before, and after, oral administration of 0.4mg /kg bodyweight D-amphetamine. Patients were followed up at regular intervals. Changes in psychopathology were quantified using the Positive and Negative Symptoms Scale. We observed significant relationships between amphetamine-induced changes in subcortical binding of the D2/3R agonist radioligand [11C]-(+)-PHNO before treatment initiation and antipsychotic response at treatment week four. In addition, we found that FEP patients who achieved remission at later points in time showed significantly larger amphetamine-induced changes in [11C]-(+)-PHNO binding before treatment initiation. Our results are in line with those of earlier studies using complementary methods for studying dopamine functioning in schizophrenia, and they support the notion that FEP patients

showing larger dopaminergic alterations may be the ones who profit more from antipsychotic treatment.

1-C-19 Functional characterization of prefrontal cortical D2 dopamine receptor in adult mice

Clémentine Quintana[1], Jivan Khlghatyan[2], Martin Beaulieu[1]
[1]University of Toronto, [2]University Laval

The dopamine D2 receptor (DRD2) remains the principal target of antipsychotic drugs used for the management of schizophrenia and other psychotic disorders. Early studies have been shown the presence of DRD2 in several cortical areas. Cortical DRD2 has been at the centre of interest because of its involvement in regulation of cognitive, emotional and social behavioural processes and its regulation by antipsychotic drugs. However, further investigations of cortical DRD2 functions have been hindered by relatively low receptor expression, antibodies and ligand selectivity or limits of gene reporter systems. We used high sensitivity approaches to map cortical DRD2 expressing neurons and its projections. Results from these investigations revealed highly heterogeneous expression of DRD2 in principal neurons and various populations of interneurons which are not exclusively expressed on parvalbumin positive interneurons. We then explored the functional role of DRD2 in prefrontal cortex (PFC) by using somatic CRISPR/Cas9 mediated knockout of DRD2 to investigate the behavioral responses of DRD2 regulation in a cell type and circuit defined manner in adult mice. Chemogenetic manipulation of DRD2 expressing neurons discriminates the role on its involvement of neuronal activity. This comprehensive analysis of PFC DRD2 expressing neurons provides indications for its functional implications in healthy and disease conditions and paves the way for a re-examination of cortical DRD2 functions, which could provide information about neuronal circuits involved in psychotic and mood disorders.

D - Dopamine, Parkinson's Disease, and neurodegeneration

1-D-20 Dopamine receptors are not implied in sleep related epilepsy in Tg2576 mice models of Alzheimer disease

Anna Szabo[1], Lionel Dahan[1]
[1]Université Paul Sabatier - Toulouse 3

Mouse models of Alzheimer disease exhibit interictal spikes and seizures during sleep and predominantly during REM sleep. We previously showed that dopamine neurons are highly active during REM sleep and others have suggested that an increase in dopaminergic transmission mediated by D1 receptors might be involved in excitation/inhibition imbalance in mouse models of Alzheimer disease. In order to test for a role of dopamine transmission in the peculiar form of epilepsy exhibited by Tg2576 during REM sleep, we recorded the EEG and EMG of these mice before and after the injection of antagonists of D1 or D2 receptors. We found that, albeit both treatments had the expected effects on the sleep-wake patterns of the animals, none of them were efficient in reducing epileptiform activity. Our results indicate that the dopaminergic transmission does not contribute significantly the epilepsy in Tg2576 mice.

1-D-21 Neuronal hemoglobin induces loss of dopaminergic neurons in mouse substantia nigra, cognitive deficits and cleavage of endogenous α-synuclein

Chiara Santulli[1], Bon Carlotta[2], Elena De Cecco[1], Marta Codrich[1], Joanna Narkiewicz[1], Pietro Parisse[3], Fabio Perissinotto[3], Claudio Santoro[4], Francesca Persichetti[4], Giuseppe Legname[1], Stefano Gustincich[2],

Stefano Espinoza[2]
[1]Scuola Internazionale Superiore di Studi Avanzati, [2]Istituto Italiano di Tecnologica, [3]ELETTRA Synchotron Light Source, [4] Department of Health Sciences and Research Center on Autoimmune and Allergic Diseases (CAAD)

Parkinson's disease (PD) presents the selective loss of A9 dopaminergic (DA) neurons of Substantia Nigra pars compacta (SNpc) and the presence of intracellular aggregates called Lewy bodies. α-synuclein (α-syn) species truncated at the carboxy terminal (C-terminal) accumulate in pathological inclusions and promote α-syn aggregation and toxicity. Hemoglobin (Hb) is the major oxygen carrier protein in erythrocytes. In addition, Hb is expressed in A9 DA neurons where it influences mitochondrial activity. Hb overexpression increases cells' vulnerability in a neurochemical model of PD in vitro and forms cytoplasmic and nucleolar aggregates upon short-term overexpression in mouse SNpc. In this study, α and β-globin chains were co-expressed in DA cells of SNpc in vivo upon stereotaxic injections of an Adeno-Associated Virus isotype 9 (AAV9) and in DA iMN9D cells in vitro. Long-term Hb over-expression in SNpc induced the loss of about 50% of DA neurons, a mild motor impairment and deficits in recognition and spatial working memory. Hb triggered the formation of endogenous α-synuclein C-terminal truncated species. Similar α-syn fragments were found in vitro in DA iMN9D cells over-expressing α and β- globins when treated with pre-formed α-syn fibrils. Our study positions Hb as a relevant player in PD pathogenesis for its ability to trigger DA cells' loss in vivo and the formation of C-terminal α-synuclein fragments.

1-D-22 Amphetamine-mediated dopamine release in the ventral striatum is associated with impulsivity in Parkinson´s disease

Alexander Song[1], Daniel Claassen[1]
[1]Vanderbilt University Medical Center

Dopamine replacement therapy in Parkinson´s disease (PD) patients can result in the development of impulsive-compulsive behaviors (ICBs). Dopamine agonists (DAA), which preferentially target D2/3 receptors, is the main risk factor for these behaviors. We have previously reported an increased DAA-mediated change of cerebral blood flow in the ventral striatum of ICB-positive PD patients, and altered ventral striatal D2/3 receptor levels, as assessed by [18F]fallypride. Here, we performed a randomized single-blind study design assessing placebo or oral administration of 0.43 mg/kg of d-amphetamine (dAMPH), where patients completed [18F]fallypride PET studies in each session. 12 PD patients completed these scans, as well as behavioral assessments which include the Barratt Impulsiveness Scale-11 (BIS-11) and Questionnaire for Impulsive-Compulsive Disorders in Parkinson´s Disease-Rating Scale (QUIP-RS). In each PET study, participants completed a 36-hour withdrawal from DAA medication. A whole brain voxel-wise evaluation revealed significant dAMPH-mediated BPND reductions in clusters ($p_{voxel}<0.005$; size>10; $q_{FDR-corr}<0.1$) localized to the ventral striatum and orbitofrontal cortex. Region-of-interest analyses revealed PD patients had significantly greater DA release in the ventral striatum than in the putamen ($p=0.003$). The extent of DA release in the ventral striatum was also highly correlated with BIS-11 motor subfactor ($r=0.663$, $p=0.019$) and QUIP-RS total scores ($r=0.839$, $p=0.001$). This finding supports the finding that DA release in the ventral striatum is the main mediator of ICBs. DAA therapy appears to alter DA regulation in the ventral striatum, resulting in the clinical manifestation of these behaviors.

1-D-23 Early synaptic and mitochondrial dysfunction caused by alfa-synuclein accumulation precedes dopaminergic neurodegeneration in a mouse model of Parkinson's Disease

A Sanz-Magro[1], N Granado[1], MG Spillantini[2], Rosario Moratalla[1]
[1]Instituto Cajal, CSIC, [2]University of Cambridge

Parkinson´s disease is the most common motor neurodegenerative disorder, caused by the loss of dopaminergic neurons of the susbtancia nigra and their terminals in the striatum. The main neuropathological signature of Parkinson´s disease is the deposit of the presynaptic protein alpha-synuclein, that compromise neuronal homeostasis and viability. These alterations are already present at early stages of the disease and are evident earlier in the synaptic terminals than in the cell bodies, indicative of the central role of synaptic failure in Parkinson´s disease. The alpha-synuclein deposits are found in different neuronal population, and results especially toxic in dopaminergic neurons, although the early pathological processes that precedes neurodegeneration in PD is not completely understood and it is capital to identify early markers of the disease. The main goal of this study is to determine the early pathological processes caused by alfa-synuclein accumulation leading to dopamine cell death. For that, we use the haSyn1-120 mouse model, that specifically accumulates a truncated form of the human alfa-synuclein in dopaminergic neurons. We found that the progressive accumulation of alpha-synuclein in striatal dopaminergic terminals induce early synaptic failure (reduced levels of vesicular monoamine transporter type 2 and synaptobrevin 2) and disrupts dopamine management. Also, alfa synuclein accumulation causes profound alterations in mitochondrial dynamics, compromising fusion/fission cycles, mitophagy and oxidative phosphorylation. Both, dopamine and mitochondrial dysregulation potentiate oxidative stress levels and inflammatory response in the nigroestriatal pathway that, in the end, causes the loss of dopaminergic terminals in the striatum at advanced ages, when motor alterations appear.

1-D-24 D2-like receptor expression in the hippocampus and amygdala informs performance on the stop-signal task in Parkinson's disease

Leah Mann[1], Kaitlyn Hay[1], Alexander Song[1], Steven Errington[1], Paula Trujillo[1], David Zald[2], Yan Yan[1], Hakmook Kang[1], Gordon Logan[3], Daniel Claassen[1]
[1]Vanderbilt University Medical Center, [2]Rutgers University, [3]Vanderbilt University

The stop-signal task serves as the benchmark assessment for measuring response inhibition, and in healthy humans, proficiency has been correlated with dorsal striatum D2 receptor availability. Parkinson's disease (PD), with depleted dopaminergic innervation to the dorsal striatum, is characterized by changes to efficiency of response inhibition. We studied 17 PD patients (6 female and 11 male) using the stop-signal paradigm in a single-blinded D-amphetamine (dAMPH) study. Participants completed [18F]fallypride positron emission topography (PET) imaging in two conditions, following either placebo or dAMPH administration. A voxel-wise analysis of the relationship between binding potential (BPND) and stop-signal reaction time (SSRT) revealed that faster SSRT is associated with greater D2-like BPND in the amygdala and hippocampus (right cluster qFDR-corr = 0.026, left cluster qFDR-corr = 0.002). A region of interest (ROI) examination confirmed this association in both the amygdala (coefficient = 248.26, p = 0.005) and hippocampus (coefficient = 2104.94, p = 0.007). The emergence of these correlations suggests a role for limbic areas in inhibitory control in this clinical population. As healthy dopaminergic systems in the dorsal striatum appear to regulate response inhibition, we interpret our findings in PD to indicate either nigrostriatal damage unmasking a mesolimbic contribution, or a compensatory adaptation from the limbic and mesial temporal dopamine systems. These novel results expand the conceptualization of action-control networks, whereby limbic and motor loops may be functionally connected.

1-D-25 Movement initiation-related functional diversity of dopamine substantia nigra neurons in mice during open-field locomotion

Daniela Schenkel[1], Marie Kuhn[1], Sebastian Betz[1], Pascal Vogel[1], Gaby Schneider[1], Jochen Roeper[1]
[1]Goethe University Frankfurt

Dopamine substantia nigra (DA SN) neurons are a major target of Parkinson disease (PD) and their loss is thought to contribute to the motor impairments in PD. Da Silva et al. (2018) demonstrated that subpopulations of DA SN neurons either increased or decreased their firing rates shortly before movement initiations. However, the functional topography of this diversity among DA neurons across the SN has not been fully characterized. We performed chronic multi-electrode recordings of pharmacologically identified DA SN neurons in awake freely-moving mice, while simultaneously tracking their head and body movements. Overall, our data set of n=59 (N=16) DA SN neurons was in accordance with the results by Da Silva and colleagues (2018) with about 30% of DA SN neurons (n=17/59) transiently increasing their firing rate (baseline to maximum: +5.3 ± 4.8Hz, mean ± SD) and also about 30% of DA SN neurons (n=18/59) transiently decreasing their firing rate (baseline to minimum: -3.6 ± 1.2Hz, mean ± SD) shortly before initiation of self-paced movement in the open field. A more fine-grained topographical analysis revealed that DA neurons with transient rate reductions were predominantly found in the medial SN (n=11/22) compared to central SN (n=7/30). These responses were absent in the lateral SN (n=0/7). In contrast, the proportion of DA neurons with transient rate increases were more prominent in central SN (n=13/30) compared to medial SN (n=3/22) and lateral SN (n=1/7). In light of the functional topography of axonal projections of DA SN neurons (Farassat et al., 2019), our data suggest a differential involvement of distinct nigrostriatal projections in self-paced movement initiation. However, definitive experiments require selective molecular tagging of distinct DA SN projections.

1-D-26 Differential gene expression analysis in midbrain organoid models of Parkinson's Disease using single cell RNA sequencing

Rhalena Thomas[1], Meghna Mathur[1], Vanessa Omana[1], Julien Sirois[1], Thomas Durcan[1], Edward Fon[1]
[1]MCGILL UNIVERSITY

More than 10 million people worldwide are living with Parkinson's Disease (PD), a central nervous system disorder that leads to dopaminergic neuron cell death. Currently no disease modifying therapies or pre-symptomatic biomarkers are available. To develop effective treatments, the early mechanisms underlying PD development must be investigated. Studying genes associated with inherited PD has great potential to reveal mechanisms underlying the development of disease both in familial and sporadic PD. Human midbrain organoids (hMOs) were grown from induced pluripotent stem cells (iPSCs) and single cell RNA sequencing was performed on three monogenic models of PD (PINK1KO, ParkinKO and SNCA triplication) with genetically matched controls. The transcriptomic data was processed using the 10X Cell Ranger pipeline, followed by custom filtering and normalization. Using Louvain network detection, groups of cell types were identified. We found that the midbrain organoids contain progenitor cells, neural precursors, neurons, radial glia, immature oligodendrocytes, and astrocytes. We identified subpopulations of neurons, two of which were enriched for markers of dopaminergic neurons. Differential gene expression (DGE) analysis followed by pathways and ontology analysis reveals changes to numerous cellular pathways. Overall, the SNCA triplication hMOs have upregulated ribosomal assembly and transcriptional activation and down-regulated mitochondrial and energy production pathways. The ParkinKO and PINK1KO hMOs upregulated cytoskeleton reorganization and

differentiation and down-regulated inhibition of peptidase and endonuclease activity pathway. We analyzed DGE in each cell population and found activation of separate molecular pathways in neuronal cells compared to glia populations,

1-D-27 Effects of age and sex on D2-autoreceptor inhibition in dopamine neurons of the substantia nigra

Eva Troyano-Rodriguez[1], Kylie Handa[1], Tamar Cohen-Davidyan[1], Michael Beckstead[1]
[1]Oklahoma Medical Research Foundation

Dopamine neurons in the substantia nigra pars compacta (SNc) are key in regulating movement and reward learning, and dopamine function declines with advanced age. SNc dopamine cells communicate with each other through dendrodendritic synapses and are under the inhibitory control of dopamine D2-type autoreceptors located throughout the somatodendritic compartment. Released dopamine activates these receptors, opening a G protein-activated potassium (GIRK) channel, causing a hyperpolarizing current, and strongly inhibiting cell firing. In the absence of synaptic input, dopamine neurons fire in a pacemaking mode, and we previously found that aging disrupts this regularity in mice, but only in males. The aim of the present study is to analyze how dopamine neurotransmission in SNc changes with age in both sexes using mouse brain slices. Our results show that the amplitude of D2-receptor inhibitory postsynaptic currents (D2-IPSCs) is moderately reduced in recordings from aged males. Local application of dopamine revealed a reduction in the amplitude of the D2-receptor currents in old males compared to young, pointing to a postsynaptic mechanism that did not extend to GABAB receptor-mediated GIRK currents. There were no age or sex differences observed in the kinetics of D2-IPSCs, and no differences in potentiation of D2-IPSCs induced by the stress neuropeptide corticotrophin releasing factor (CRF). These findings are important to understand dopamine transmission during aging and have implications for the development of therapeutic strategies for treating age-related disorders.

1-D-28 Effect of the reversible inhibitor of monoamine oxidase A moclobemide as monotherapy on parkinsonism in the MPTP-lesioned marmoset model of Parkinson's disease

Philippe Huot[1], Stephen Nuara[1], Jim Gourdon[1], Adjia Hamadjida[1]
[1]McGill University

While monoamine oxidase (MAO) type B inhibitors are used to treat motor symptoms of Parkinson disease, MAO type A (MAO-A) inhibitors are not used for this indication. Because both MAO-A and MAO-B participate in the deamination of dopamine, MAO-A inhibition might lead to higher dopamine levels within the striatum, suggesting that it could elicit an anti-parkinsonian effect. Here, we assessed the effect of the clinically-available reversible MAO-A inhibitor moclobemide as monotherapy on parkinsonism in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset. Six marmosets were rendered parkinsonian by MPTP administration. Following repeated administration of L-DOPA to induce stable dyskinesia and psychosis-like behaviours (PLBs), marmosets were administered acute challenges of moclobemide (0.1 and 1 mg/kg) or vehicle, after which the severity of parkinsonism, dyskinesia and PLBs was rated by a blinded observer. Marmosets were also administered a therapeutic dose of L-DOPA, so that the anti-parkinsonian effect of moclobemide could be compared to that of L-DOPA. We found that moclobemide (0.1 and 1 mg/kg) significantly increased the duration of on-time by ≈ 5.71 folds (12 min vs 78 and 68 min, $P < 0.05$ and $P < 0.01$, respectively), while L-DOPA showed a ≈ 11.57 folds increase when compared to the vehicle (146 min, $P < 0.001$). Unlike L-DOPA, the anti-parkinsonian action conferred by moclobemide was achieved without inducing complications such as

dyskinesia and PLBs. These results suggest that moclobemide, a clinically-available reversible MAO-A inhibitor, could potentially be repurposed to reduce the severity of parkinsonism, without associated dyskinesia or psychotic behaviours.

1-D-29 The role of dopamine in risk-promoting effects of reward-paired cues: A study in Parkinson's disease patients

Mariya Cherkasova[1], Tanya Feng[1], Pratibha Surathi[2], Shanna C. Yang[1], Martin J. McKeown[1], Sike Appel-Cresswell[1], Catharine Winstanley[1], A. Jon Stoessl[1], Jason JS Barton[1]
[1]University of British Columbia, [2]Harlem Hospital Center

Reward-paired sensory cues have been found to promote riskier choice in both rodents and humans. In rodents, these effects are dopamine (DA) dependent, but the neural substrates in humans have remained unknown. Here, our objective was to investigate the role of dopamine in promoting cue-induced risky choice in humans by examining the modulation of these effects by DA replacement therapy in Parkinson's disease (PD) patients. We tested 39 patients with idiopathic PD; 21 were treated with levodopa monotherapy and 18 were treated with a combination of levodopa and DA agonists. The PD patients and 13 healthy controls performed two versions of a risky decision-making task previously validated in healthy volunteers. In one version of the task, rewards were accompanied by images of money and casino jingles; in the other version, rewards were unaccompanied by the reward cues. Patients performed both task versions ON and OFF their usual DA medication, in a randomized order. Healthy controls performed both versions twice. Overall, patients on levodopa monotherapy were risk-averse relative to the patients additionally treated with DA agonists, who did not differ in their risk attitudes from controls. Reward-paired sensory cues promoted riskier choice in both the healthy controls and the PD patients OFF medication and ON levodopa. However, dopamine agonists abolished the risk-promoting effect of reward-paired cues. These findings support the role of dopamine in the risk-promoting effects of reward-paired cues in humans. Direct DA receptor agonism may have abolished these risk-promoting effects by interfering with endogenous patterns of synaptic DA signalling. These endogenous signals might be necessary for enabling cue-responsivity of decision making.

1-D-30 Combining ngn2 programming and dopaminergic patterning for a rapid and efficient generation of hiPSC-derived midbrain neurons

Razan Sheta[1], Maxime Teixeira[1], Walid Idi[1], Marion Pierre[1], Vincent Emond[1], Cornelia Zorca[2], Benoît Vanderperre[3], Thomas Durcan[2], Edward Fon[1], Frédéric Calon[1], Mohamed Chahine[1], Abid Oueslati[1]
[1]ULaval, [2]McGill University, [3]Université du Québec à Montréal

Objective: The use of human derived induced pluripotent stem cells (hiPSCs) differentiated to dopaminergic (DA) neurons offers a valuable experimental model to decorticate the cellular and molecular mechanisms of Parkinson's disease (PD) pathogenesis. However, the existing approaches present with several limitations, notably the lengthy time course of the protocols and the high variability in the yield of DA neurons. Methods and results: Here we report on the development of a new approach that combines neurogenin-2 programming with the use of commercially available midbrain differentiation kits for a rapid, efficient, and reproducible directed differentiation of hiPSCs to mature and functional induced DA (iDA) neurons. Gene expression analysis, associated with functional characterization examining neurotransmitter release and electrical recordings, support the functional identity of the iDA neurons to A9 midbrain neurons. iDA neurons also showed selective vulnerability when exposed to 6-hydroxydopamine, thus providing a viable in vitro approach for modelling PD and

related disorders. Conclusions: The DA differentiation protocol we designed here offers attractive prospects for accelerated specification of DA neurons while significantly reducing the expense of obtaining DA neurons of high purity.

E – Developmnet and diversity of the dopamine systems

1-E-31 Spatial registration of gene expression in the human locus coeruleus

Lukas Weber[1], Heena Divecha[2], Sang Ho Kwon[2], Matt Tran[2], Madhavi Tippani[2], Abby Spangler[2], Kelsey Montgomery[2], Thomas Hyde[2], Stephanie Page[2], Leonardo Collado-Torres[2], Kristen Maynard[2], Stephanie Hicks[1], Keri Martinowich[2]

[1]Johns Hopkins University, [2]Lieber Institute for Brain Development

The locus coeruleus (LC) is a brainstem nucleus that is the primary brain site for production of the neuromodulator norepinephrine (NE). LC-NE neurons project widely throughout the brain, playing important roles in diverse behaviors and cognitive function. Early emergence of pathology and resulting loss of LC neurons is a hallmark in Alzheimer's and Parkinson's disease. Evidence also suggests that maintaining the integrity of the LC-NE system plays a key role in cognitive abilities in later life. Understanding cellular diversity and molecular composition of the LC in the human brain could provide insight into why LC-NE neurons are particularly vulnerable to aging and neurodegeneration. To molecularly characterize the human LC, we generated single-nucleus and transcriptome-scale spatial gene expression data from four neurotypical brain donors using the 10x Genomics single-cell and Visium spatial transcriptomics platforms. Using data-driven approaches, we identified spatially variable genes across the LC, and investigated enrichment of genes associated with Alzheimer's and Parkinson's disease in LC populations. We used single-nucleus RNA-sequencing data and manually annotated Visium data to create reference expression profiles for LC-NE neurons and other resident cell populations to improve Visium spot-level deconvolution in spatial transcriptomics data. In summary, we define the spatial topography of gene expression in human LC and identify molecular profiles for LC-NE neurons. These cell-type reference profiles can be used to better understand links between LC gene expression and neurodegenerative and neuropsychiatric disease that are associated with LC-NE signaling, which could facilitate the ability to target this population for disease prevention and treatment.

1-E-32 The role of Netrin-1 the Development of Inhibitory Control in Females

Del MacGowan[1], Claire Guyatt[1], Giovanni Hernandez[1], Cecilia Flores[1]

[1]McGill University

The Netrin-1 guidance cue mediates axon targeting in the nucleus accumbens (NAc) in adolescence, controlling dopamine axon growth to prefrontal cortex (PFC) until early adulthood. In male mice, amphetamine (AMPH) in adolescence downregulates NAc Netrin-1, disrupting adult PFC DA connectivity and cognitive control. Female mice appear insensitive to these effects. Here we characterized NAc Netrin-1 expression in adolescent females and determined whether Netrin-1 silencing during this period alters impulsivity in adulthood. Netrin-1 expression in dopamine D1 and/or D2 NAc neurons was characterized using RNAscope in adolescent C57BL/6 mice (n=3, PND35). A separate cohort (n=30) received bilateral microinjections of either a Netrin-1 shRNA or scrambled lentivirus into the NAc at PND21. Two weeks later, mice received non-contingent injections of a recreational-like dose of AMPH (4mg/kg, i.p.) or saline, every second day, for 10 days. In adulthood, wait and stop impulsivity were assessed using the Go/No-Go task. Netrin-1 is highly expressed in NAc D1 and D2-positive neurons. Administration of the Netrin-1 shRNA does not alter amphetamine-induced locomotion in adolescence.

In adulthood, mice administered the scrambled virus and exposed to AMPH in adolescence show greater wait impulsivity than saline controls (increased premature responses). This effect is prevented in mice with NAc Netrin-1 knockdown. Adult stop impulsivity is improved in mice with Netrin-1 knockdown, independent of adolescent drug treatment (reduced commission errors compared to control). In females, Netrin-1 appears to mediate AMPH-induced deficits in stop impulsivity and influence the normative development of wait impulsivity. These changes may be due to altered mesocorticolimbic dopamine development.

1-E-33 Development of dopaminergic axon arbors

Laurens Grossouw[1], Oxana Garritsen[1], Mateja Rybiczka-Tesulov[1], Youri Adolfs[1], Mark Broekhoven[1], Eljo Van Battum[1], Jeroen Pasterkamp[1]
[1]Utrecht University

The midbrain dopamine (mDA) system projects towards the striatum via the mesostriatal pathway as part of the medial forebrain bundles. mDA neurons are organized into distinct regions, including the substantia nigra pars compacta (SNc), ventral tegmental area (VTA) and retro-rubral field (RRF). Within the striatum, SNc neurons project their axons primarily towards the dorsolateral striatum, while VTA neurons project predominantly towards the ventromedial striatum. In adult mice, mDA neurons form widespread and dense axonal arbors throughout the striatal patch- and matrix compartments. Understanding the characteristics of the axonal arbors of SNc and VTA neurons will help to understand the disease mechanisms underlying disorders such as Parkinson's disease. Tracing of individual mDA neurons classically relies on viral tracers, which precludes studies on axonal arbors during embryonic and early postnatal stages. Here, we used an intersectional genetics approach in combination with 3D light sheet microscopy to sparsely label and visualize mDA neurons and their axonal projections during development. Pitx3-Flp;Gucy2c-Cre;Ai65 mice express Tdtomato in a subset of mDA neurons. Brains from Pitx3-Flp;Gucy2c-Cre;Ai65 mice were whole-mount immunostained, cleared using iDISCO, and imaged using a lightsheet microscope. Axon branching is first observed in the dorsolateral striatum at E17.5, while in the ventromedial striatum it is delayed until E18.5. mDA axonal arbors rapidly increase in complexity at late embryonic and early postnatal stages of development. These data provide new insights into the differences between SNc and VTA axonal arbors. Further, our work provides tools and a framework for further understanding dopaminergic axon branching in health and disease.

G – Imaging Dopamine

1-G-34 Dopamine spatiotemporal dynamics comparison between members of the dLight sensors family

Julie Chouinard[1], Akash Pal[2], Sakiko Takahashi[1], Kiyoto Kurima[1], Nobuyoshi Kitamura[1], Lin Tian[2], Jeffery Wickens[1]
[1]Okinawa Institute of Science and Technology, [2]University of California Davis

The growing toolkit of genetically encoded fluorescence biosensors opens new avenues for studying the spatiotemporal dynamics of neurochemical signaling in the brain. These sensors directly report synaptic release with specificity and high resolution. dLight1 is a family of dopamine (DA) sensors based on three inert human DA receptors with broadly tuned apparent affinity and dynamic range suitable to measure DA release between pM and µM range. However, the intrinsic properties of sensors such as expression level and patterns, apparent affinity, kinetics, and dynamic range, can profoundly affect signal-to-noise ratio (SNR) for in vivo applications that is brain-region and cell-type specific. We developed a viral-based

strategy to maximize the SNR of DA imaging in various brain regions. We reported an optimized dLight, dLight2.1, with improved SNR and compared its performance with dLight1.3b and RdLight driven by various viral vectors in both mPFC and striatum. We also compared the performance of DA imaging with simultaneously recorded fast-scan voltammetry traces with electrical stimulation with and without the DA reuptake inhibitor methylphenidate. When sensors are expressed perisynaptically, they appear to report dopamine levels near release sites, whereas voltammetry detects DA that has diffused some distance. A comparison between sensors revealed differences in dopamine levels and spatiotemporal dynamics that are dependent on expression level and pattern. Our data show that dLight sensors can pave the way toward a more complete understanding of neurotransmitter dynamics in the basal ganglia circuitry and beyond. With improved imaging and analysis methods, these biosensors could be useful tools to decipher neural activity into its composite molecular signaling events.

1-G-35 [11C]-(+)-PHNO Bolus + Infusion: A novel tool to quantify dopamine release in extra-striatal brain regions

Ulrich Sauerzopf[1], Ana Weidenauer[1], Irena Dajic[1], Irene Graf[1], Martin Bauer[1], Lukas Nics[1], Cecile Philippe[1], Sarah Pfaff[1], Markus Mitterhauser[1], Wolfgang Wadsak[1], Rupert Lanzenberger[1], Nicole Praschak-Rieder[1], Matthäus Willeit[1]

[1]Medical University of Vienna

(+)-4-propyl-9-hydroxynaphthoxazine labled with carbon-11 ([11C]-(+)-PHNO) is a D3 receptor (D3R) preferring D2/3 R agonist radioligand that is easily displaceable by endogeneous DA. Most extra-striatal regions display too little specific binding to estimate binding potential values (BPND) and thus quantification of DA release. Extrastriatal DA release, is of great interest in the research into major psychiatric disorders. Five healthy volunteers underwent two identical [11C]-(+)-PHNO PET employing a bolus + constant infusion paradigm. After radioligand equilibration, subjects were challenged with d-amphetamine (AMPH) intravenously. Radioligand displacement was quantified using the ratio method. These data were compared to five matched volunteers scanned twice using a bolus after an oral challenge with AMPH. Data obtained with the bolus method were analyzed by contrasting of BPND values. [11C]-(+)-PHNO bolus + infusion time activity curves achieve stable equilibrium in most brain regions, including cortical regions. Accumulation of local radioactivity was observed in high binding areas of the ventral striatum and the globus pallidus. Across all equilibrating brain regions, reductions in binding were observed following AMPH challenge. Voxel-wise inference confirmed widespread radioligand displacement, indicating DA release in prefrontal and temporal cortical regions, insula, amygdala, and the hippocampus. [11C]-(+)-PHNO bolus+infusion provides a novel tool for quantifying extra-striatal DA release. The method remains limited by the inability to quantify both, high- and low-binding regions simultaneously. Differential behavior of bolus and bolus+infusion methods regarding spatial patterns of DA release suggest more accurate representation of pharmacological effects.

1-G-36 Applications of functionalized carbon nanotubes for fluorescently imaging extracellular dopamine

Madeline Klinger[1], Abraham Beyene[1], Jackson Travis Del Bonis-O'Donnell[1], Kristen Delevich[1], Linda Wilbrecht[2], Markita Landry[2]

[1]University of California Berkeley, [2]University of California, Berkeley

Neuron-to-neuron communication can be tuned on a larger scale by neuromodulators that diffuse beyond the synaptic cleft to affect the firing of broader neuron populations. Neuromodulator signaling,

particularly dopamine, plays a key role in many neurological and psychiatric disorders. While decades of research have elucidated general roles for dopamine, how this neuromodulator impacts the function of neural networks remains largely unclear. To investigate the nuances of dopamine transmission, neuroscience requires a spatiotemporally precise and direct method to detect and quantify dopamine at the cellular scale. A promising candidate to fulfill this purpose are non-genetically-encoded, near-InfraRed Catecholamine nanosensors (nIRCats). nIRCats are intrinsically near infrared-fluorescent single-walled carbon nanotubes (SWNTs) functionalized by the adsorption of DNA polymers to the carbon surface, rendering them highly sensitive to dopamine. Our group has validated nIRCats as a reliable fluorescent detector of dopamine transmission in the striatum and prefrontal cortex, with up to ΔF/F = 4500% near-infrared fluorescence above baseline levels upon release of dopamine (Beyene et al. Nano Letters, 2018). Additionally, nIRCats permit detection of altered dopamine reuptake kinetics when brain tissue is exposed to dopamine agonist and antagonist drugs (Beyene et al. Science Advances, 2019), suggesting applicability for investigation of psychiatric disease. While as to date nIRCats have been validated only in ex vivo brain tissue, these initial applications implicate nIRCats as a novel tool for direct investigation of the spatiotemporal dynamics of neuromodulatory signaling in vivo. We here present challenges and opportunities for use of this tool in the living brain.

1-G-37 Dopamine release induced by d-amphetamine is mediated by sex hormones: A [11C]-(+)-PHNO study in healthy female subjects

Ana Weidenauer[1], Ulrich Sauerzopf[1], Carina Bum[1], Martin Bauer[1], Lucie Bartova[1], Cecile Philippe[1], Sarah Pfaff[1], Neydher Berroteran-Infante[1], Verena Pichler[1], Lukas Nics[1], Markus Mitterhauser[1], Wolfgang Wadsak[1], Rupert Lanzenberger[1], Siegfried Kasper[1], Nic
[1]Medical University of Vienna

Sex differences in dopamine-related disorders such as schizophrenia, Parkinson's disease, or addiction, led to the conclusion that sex-specific neurodevelopment and sex hormones have a substantial impact on brain dopamine functioning. Positron-emission-tomography (PET) allows for investigating these differences in the living human brain. Using PET, an increased reactivity of the dopamine system after pharmacological challenges has been shown in males, while in females an important impact of estrogen and progesterone on behavioral and neurochemical responses was observed. To increase the knowledge on these interactions, we studied fourteen healthy females (25.9 ±2.8 years, eight subjects on hormonal estrogen/progesterone combination contraceptives) and exposed them to oral d-amphetamine (0.4 mg/kg bodyweight). We measured d-amphetamine-induced dopamine release by performing 90-minute PET scans using the dopamine D2/3 receptor agonist radioligand [11]-(+)-PHNO under drug-naïve and under amphetamine conditions. Sex hormones (follicle stimulating hormone; FSH, luteinizing hormone; LH, estrogen, progesterone, and testosterone were assessed in blood serum. Behavioral responses to d-amphetamine were recorded using designated questionnaire. We found a positive relationship between estrogen levels and behavioral effects of d-amphetamine ($p<0.05$). On a neurochemical level, FSH, LH and estrogen levels correlated positively with d-amphetamine-induced dopamine release in the globus pallidus. A decrease in d-amphetamine-effects was detected in females taking hormonal contraception (two-sided t-test, $p<0.05$). We conclude that investigating the impact of sex hormones can give meaningful insights into the neurobiological basis of sexual dimorphisms in dopaminergic disorders.

I – Anatomy and physiology of Dopamine systems

1-I-38 Dopamine neuron glutamate release sites in the forebrain revealed by proximity

Alyssa Martin[1], Megan Caldwell[1], Paul McKee[2], Darya Ryndych[1], Philip Fonseca[1], Clare Mitchell[1], Elizabeth

Fiore[1], Gary Shteyman[1], Bianca Field[3], Pierre Trifilieff[4], Nao Chuhma[1], Imad Antonios[5], Susana Mingote[1], Stephen Rayport[6], Leora Yetnikoff[1]
[1]City University of New York, [2]Duke University, [3]University of Texas Southwestern, [4]University of Bordeaux, [5]Southern Connecticut State University, [6]Columbia University

Dopamine neurons using glutamate as a co-transmitter elicit fast excitatory synaptic actions in the ventral striatum and slower excitation in the lateral dorsal striatum. While intersectional strategies reveal that dopamine-glutamate neurons predominantly project to the same striatal domains, with minimal projections to other forebrain regions, the distribution of dopamine neuron glutamate release sites is unknown. To identify synaptic glutamate release sites in dopamine-glutamate neuron axons, we queried proximity of glutamatergic synaptic vesicles to the dopamine neuron plasmalemma. We applied proximity ligation assay in DAT-IREScre;Ai32 mice with dopamine neuron specific expression of ChannelRhodopsin2 tagged with enhanced yellow fluorescent protein in the plasmalemma to visualize as puncta sites where glutamate synaptic vesicles, identified by glutamate transporter VGluT2, were within 20 nm of the plasmalemma, as putative release sites. Mapping the distribution of puncta across the forebrain in coronal sections revealed that a high density of puncta coincided with the striatal regions with dopamine-glutamate neuron evoked excitatory responses and projections, but also throughout the striatum, most notably in the caudal medial dorsal striatum, and also in the post-genum cingulate cortex and bed nucleus of the stria terminalis. Sparser puncta were seen in other striatal and forebrain regions, consistent with a role for VGluT2 in mediating vesicular synergy in most dopamine neurons. This approach prototypes a way to visualize neurochemically defined synaptic release sites in genetically defined neurons comprehensively.

1-I-39 Selective intrinsic bursting in dopamine VTA neurons projecting to medial shell of Nucleus accumbens

Tabea Ziouziou[1], Christopher Knowlton[2], Carmen Canavier[2], Jochen Roeper[1]
[1]Goethe University, Frankfurt, Germany, [2]Louisiana State University Health Sciences Center

The functional diversity of the mesolimbic dopamine system comprises relevant differences in biophysical properties such as e.g. the dynamic range of firing and rebound properties in a projection-specific manner (Lammel et al. 2008, Knowlton, Ziouziou et al. 2021). Based on the assumption that mature and synaptically isolated DA neurons do not spontaneously generate burst discharges in vitro, this analysis has so far been focused on single spike firing patterns. However, by combining retrograde tracing and in vitro perforated or on-cell patch-clamp recordings, we recently identified a small subset of medial shell of Nucleus accumbens-projecting (mNAcc) DA VTA neurons that spontaneously generate high-frequency burst discharges. Stimulation of muscarinic receptors by 1 µM Oxotremorine M increased the occurrence of bursting in mNAcc-projecting DA VTA neurons to about 30% (n = 7/16 perforated patch; n = 6/21 on-cell) but did not induce any bursting in lateral shell of Nucleus accumbens-projecting DA neurons (n = 0/6 perforated patch; n = 0/12 on-cell). The burst properties of mNAcc DA VTA neurons were reminiscent of recently observed in vivo plateau bursting (Otomo et al. 2020) with 2-4 high frequent spikes and a significant depolarizing shift in action potential thresholds (intraburst frequency: 45.9 ± 28.6 Hz; spikes in bursts: 2.4 ± 0.7; threshold shift between 1st and last intraburst spikes: 4.4 ± 3.8 mV; n = 7, N = 9; mean ± SD). We are currently modelling these bursts using our recently developed subpopulation-specific DA computer models to identify biophysical candidate mechanisms for experimental validation. Our results might relate to previous work on cholinergically-stimulated in vivo bursting of mesolimbic DA VTA subpopulations (Dautan et al. 2016).

1-I-40 A role for the netrins in survival of subpopulations of midbrain dopaminergic neurons

Abbas Sadikot[1], Pik-Shan Lo[1], Vladmir Rymar[1], Gabriella Kennedy[1], Timothy Kennedy[1]
[1]Montreal Neurological Institute, McGill

Mechanisms that determine the survival of midbrain dopaminergic neurons (mDAs) in the adult central nervous system (CNS) are not fully understood. In the mature CNS, mDAs express particularly high levels of netrin-1 and its receptor Deleted in Colorectal Cancer (DCC). Recent findings indicate that overexpressing netrin-1 protects mDA neurons in animal models of Parkinson's disease (PD), with a proposed pro-apoptotic dependence function for DCC that triggers cell death in the absence of ligand. Here we sought to determine if DCC expression influences mDA survival in young adult and ageing mice. To circumvent the perinatal lethality of DCC null mice, we selectively deleted DCC from mDAs utilizing DATcre/loxP gene-targeting and examined neuronal survival in adult and aged animals. Reduced numbers of mDAs were detected in the substantia nigra pars compacta (SNc) of young adult DATcre/DCCfl/fl mice, with further reduction in aged DATcre/DCCfl/fl animals. In contrast to young adults, aged mice also exhibited a gene dosage effect, with fewer SNc mDA neurons in DCC heterozygotes (DATcre/DCCfl/wt). Notably, loss of mDA neurons in the SN was not uniform. Neuronal loss in the SN was limited to ventral tier mDA neurons, which are vulnerable in PD. In the ventral tegmental area (VTA), young adult mice with conditional deletion of DCC had normal mDA neuronal numbers, while significant loss occurred in aged DATcre/DCCfl/fl and DATcre/DCCfl/wt mice compared to age-matched wild-type mice. A comparison with Netrin-1 expression in mDA neurons revealed widespread localization in the SN and VTA. Our results indicate that expression of DCC is required for the survival of subpopulations of mDA neurons and may be relevant to the degenerative process in PD.

J – Dopamine and brain circuitry

1-J-41 Inhibitory control of dopamine neurons

Rebekah Evans[1], Zayd Khaliq[1]
[1]National Institutes of Health

Substania nigra (SNc) dopaminergic neurons show a pause-rebound firing pattern in response to aversive events. Because these neurons integrate information from predominately inhibitory brain areas, it is important to determine which inputs functionally inhibit the dopamine neurons and whether this pause-rebound firing pattern can be produced by a solely inhibitory input. Here, we functionally map genetically-defined inhibitory projections from the dorsal striatum (striosome and matrix) and globus pallidus (GPe; parvalbumin and Lhx6) onto SNc neurons. We find that GPe and striosomal inputs both pause firing in SNc neurons, but rebound firing only occurs after inhibition from striosomes. Indeed, we find that striosomes are synaptically optimized to produce rebound and preferentially inhibit a subpopulation of ventral, intrinsically rebound-ready SNc dopaminergic neurons on their reticulata dendrites. Therefore, we describe a self-contained dendrite-specific striatonigral circuit that can produce pause-rebound firing in the absence of excitatory input.

1-J-42 Striatal glycine inhibits axonal dopamine release in a region-specific manner and partly via regulation of striatal ACh

Rishi Anand[1], Stefania Vietti-Michelina[1], Katherine Brimblecombe[1], Stephanie Cragg[1]
[1]University of Oxford

Striatal dopamine (DA) axons are key sites for gating DA release by neurotransmitters and in region-dependent manners. Prior studies have shown that ligands for glycine receptors (GlyRs) alter extracellular DA concentrations ([DA]o) but report a range of outcomes. This heterogeneity might arise from direct or indirect effects via other neurotransmitters, e.g. ACh, and from regional diversity. We explored how glycine modulates [DA]o detected in real-time using fast-scan cyclic voltammetry evoked locally in striatum in ex vivo slices from C57BL6/J mice. Glycine (5-10 mM) reduced electrically or optically evoked [DA]o and to a greater extent in dorsal than ventral striatum. Inhibition of glycine transporter 1 (GLYT1, 500 µM sarcosine) enhanced the effect of glycine, but alone did not alter evoked [DA]o. GlyR agonist taurine (10 mM) and, paradoxically, GlyR antagonist strychnine (10 µM) reduced evoked [DA]o, reflecting either agonist-mediated GlyR desensitisation or non-specific pharmacology of GlyR ligands. Glycine modulation of DA release did not involve co-agonism at NMDA receptors or modulation of GABA tone on DA axons, as antagonism of NMDA (50 µM AP-V), GABAA (10 µM bicuculline) or GABAB receptors did not alter the effect of glycine. Glycine increased the frequency-dependence of electrically evoked DA release, suggesting an action via ACh acting at nAChRs, and an nAChR inhibitor partially attenuated glycine effects. We imaged fluorescence of virally expressed GRABACh3.0 sensor and identified that glycine reduced levels of striatal ACh. To conclude, glycine reduces DA release particularly in dorsal striatum, partially via inhibition of ACh release and nAChR activation, but also partially via a mechanism consistent with putative GlyRs on DA axons.

1-J-43 Open-loop striato-nigro-striatal circuits from limbic and sensorimotor striatum trigger dopamine release in the associative striatum

Rudolf Faust[1], Louisa Linders[2], Anastasia Markidi[2], Lennard Klein[3], Jelte de Vries[4], Nicole Yee[1], Ralph Hamelink[1], Larry Zweifel[5], Frank Meye[2], Ingo Willuhn[1]

[1]University of Amsterdam, [2]Utrecht University, [3]University of Maastricht, [4]Free University of Amsterdam, [5]University of Washington

Haber and colleagues' ascending spiral hypothesis posits that the limbic, ventromedial striatum (VMS) influences associative and sensorimotor domains of the dorsal striatum through serial open striato-nigro-striatal projection loops. We sought to provide evidence for this hypothesis by dissecting how afferents from specific functional domains of striatum to the substantia nigra control dopamine release. Combining optogenetics with fast-scan cyclic voltammetry in vivo and whole-cell recording ex vivo, we interrogated candidate circuits for region-specific control of dorsal striatal dopamine release. Optogenetic stimulation of striatonigral cell bodies in, or axon terminals originating from, the VMS or anterior dorsolateral striatum (aDLS) evoked phasic dopamine release in the associative, posterior dorsomedial striatum (pDMS), but not the sensorimotor aDLS. Retrograde tracing of monosynaptic inputs to dopamine neurons projecting to pDMS or aDLS revealed that they are innervated by topologically distinct populations of substantia nigra pars reticulata non-dopamine neurons. Furthermore, striatonigral projections from VMS and aDLS exhibited greater synaptic connectivity with non-dopamine neurons in the substantia nigra than with pDMS-projecting dopamine neurons, indicating that striatonigral projections from VMS and aDLS may evoke pDMS dopamine release via the projection-specific disinhibitory mechanism hypothesized by Haber and colleagues. Together, our results are consistent with ascending and descending spirals respectively linking limbic and sensorimotor striatum with associative striatum. Our findings strengthen the evidence that open striato-nigro-striatal loops are substrates for region-specific communication between different functional domains of the striatum.

1-J-44 Functional and molecular heterogeneity of D2R neurons along dorsal ventral axis in the striatum

Emma Puighermanal[1], Laia Castell[2], Anna Esteve-Codina[3], Su Melser[4], Konstantin Kaganovsky[5], Charleine Zussy[2], Jihane Boubaker-Vitre[2], Marta gut[6], Stéphanie Rialle[2], Christoph Kellendonk[7], Elisenda Sanz[1], Albert Quintana[1], Giovanni Marsicano[4], Miquel Marti

[1]Autonomous University of Barcelona, [2]University of Montpellier, [3]Barcelona Institute of Science and Technology, [4]NeuroCentre Magendie, [5]Stanford University, [6]Universitat Pompeu Fabra, [7]Columbia University, [8]University of Buenos Aires, [9]Sorbonne Universit

Action control is a key brain function determining the survival of animals in their environment. In mammals, neurons expressing dopamine D2 receptors (D2R) in the dorsal striatum (DS) and the nucleus accumbens (Acb) jointly but differentially contribute to the fine regulation of movement. However, their region-specific molecular features are presently unknown. By combining RNAseq of striatal D2R neurons and histological analyses, we identified hundreds of novel region-specific molecular markers, which may serve as tools to target selective subpopulations. As a proof of concept, we characterized the molecular identity of a subcircuit defined by Wfs1 neurons and evaluated multiple behavioral tasks after its temporally-controlled deletion of D2R. Consequently, conditional D2R knockout mice displayed a significant reduction in digging behavior and an exacerbated hyperlocomotor response to amphetamine. Thus, targeted molecular analyses reveal an unforeseen heterogeneity in D2R-expressing striatal neuronal populations, underlying specific D2R's functional features in the control of specific motor behaviors.

1-J-58 Unexpected inhibition of motor function by dopamine activation of D1/D2 co-expressing striatal neurons

Patricia Bonnavion[1], Christophe Varin[1], Ghazal Fakhfouri[2], Aurelie De Groote[1], Amandine Cornil[1], Elsa Isingrini[2], Quentin Rainer[2], Eleni Tzavara[2], Erika Vigneault[2], Sylvie Dumas[3], Alban de Kerchove d'Exaerde[1], Bruno Giros[2]

[1]Université Libre de Bruxelles, [2]McGill University, [3]Oramacell

The central function of the striatum and its dopaminergic (DA) afference in motor control and integration of cognitive and emotional processes is commonly explained by the two striatal efferent pathways characterized by striatal projection neurons (SPNs) expressing DA D1 receptor- and D2 receptor (D1-SPNs and D2-SPNs), respectively, regardless of SPNs co-expressing these two receptors (D1/D2-SPNs). Here, after developing an approach that enables to target these hybrid SPNs, we demonstrated that, although these SPNs are rare, they play a major role in guiding the motor function of the other two main populations and convey a DA-mediated antagonistic motor brake. D1/D2-SPNs project exclusively to the external globus pallidus (GPe) and have specific electrophysiological features with distinctive integration of DA signals. Optogenetic stimulation and loss-of-function experiments indicated that D1/D2-SPNs potentiate the prokinetic and antikinetic functions of D1-SPNs and D2-SPNs, respectively, and restrain the integrated motor response to psychostimulants. Overall, our findings demonstrate the essential role of this third unacknowledged population of D1/D2 co-expressing neurons, which orchestrates the fine-tuning of DA regulation in the thalamo-cortico-striatal loops.

K – Dopamine receptors , transporters & signalling

1-K-45 Novel allosteric modulator attenuates HIV-1 Tat protein-induced inhibition of dopamine transporter and alleviates cognitive and cocaine rewarding effects in HIV-1 Tat transgenic

mice

Jun Zhu[1], Chang-Guo Zhan[2], Jay McLanghlin[3], Aam Ananthan[4], Sarah Davis[1], Corinne Augelli-Szafran[4]
[1]College of Pharmacy/University of South Carolina, [2]College of Pharmacy/University of Kentucky, [3]College of Pharmacy/University of Florida, [4]Southern Research Institute

Cocaine abuse increases the incidence of HIV-1 associated neurocognitive disorders (HAND). We have demonstrated that HIV-1 Tat protein allosterically modulates dopamine (DA) reuptake via DA transporter (hDAT), potentially contributing to Tat-induced cognitive impairment and potentiation of cocaine-conditioned place preference (CPP). This study determined whether a novel allosteric modulator, SRI-32743, ameliorates the effects of Tat binding to DAT and alleviates Tat-induced potentiation of cognitive impairment by novel object recognition (NOR) testing and cocaine reward by CPP in inducible Tat transgenic (iTat-tg) mice. SRI-32743 (50 nM) in vitro inhibited [3H]DA uptake and [3H]WIN35,428 binding, and decreased the affinity of cocaine inhibiting [3H]DA uptake in combination with cocaine compared to cocaine alone in cells expressing hDAT. SRI-32743 decreased the cocaine-induced dissociation rate of [3H]WIN35,428 binding and attenuated Tat protein-inhibited [3H]DA uptake and [3H]WIN35,428 binding. Induction of Tat expression in iTat-tg mice by a 14-day administration of doxycycline resulted in a 31.7% reduction of phase 3 recognition index in NOR and a 2.7-fold potentiation of cocaine-CPP compared to the respective vehicle-treated iTat-tg mice. Systemic administration (i.p.) of SRI-32743 prior to behavioral testing ameliorated Tat-induced impairment of NOR (at a dose of 10 mg/kg) and the Tat-induced potentiation of cocaine-CPP (at a dose of 1 or 10 mg/kg). No effect was observed in saline-treated (uninduced) iTat-tg or doxycycline-treated G-tg (Tat-null) mice. These findings validate that Tat and cocaine interaction with DAT can be modulated through an allosteric modulation manner, suggesting a potential therapeutic intervention for HAND with concurrent cocaine abuse.

1-K-46 Decoding dopamine signaling in the striatum

Andrew Yee[1], Christopher Ford[1]
[1]University of Colorado Anschutz Medical Campus

Dopamine (DA) signaling is commonly thought to be mediated by volume transmission, whereby low concentrations of the transmitter non-selectively activate extra-synaptic DA receptors after its diffusion from distant release sites. However, recent evidence has indicated that high concentrations of DA are released from sparse hotspots which comprise only a small proportion of striatal DA varicosities. Until now, existing tools have limited our ability to resolve the spatial and concentration dynamics of DA signaling. Using a combination of two-photon imaging of a genetically-encoded DA sensor and whole-cell electrophysiology to measure post-synaptic responses in striatal medium spiny neurons (D2-MSNs), we have spatially and temporally quantified electrically-stimulated DA release, and directly correlated these signals with post-synaptic responses. These responses could be reproduced by focal application of a high, but not a low concentration of exogenous DA, confirming that receptor activation requires high concentrations of DA. Highly localized stimulation of only a few DA release sites evoked spatially-restricted release along small sections of dendrites, producing quantifiable post-synaptic responses. Taken together, these data suggest that tight organization between DA release sites and the dendrites of D2-MSNs shapes post- synaptic responses, challenging the idea that DA signaling is only mediated by volume transmission.

1-K-47 Control of hippocampal theta rhythm by dopamine: role of D2 receptors in Sst and PV interneurons

Pola Tuduri[1], Emmanuel Valjent[1], Jeanne Ster[1]
[1]Institut de Génomique Fonctionnelle

Recent evidences show that dopamine is involved in hippocampal functions, notably in memory and novelty processing. These functions are underpinned by synchronous network activity. While the role of the hippocampal D1 receptor in mnesic functions has been extensively studied, the contribution of the D2 receptor (D2R) remains elusive. We characterize the functional role of D2R in the hippocampus network by combining anatomo-functional approaches. Our in situ hybridization analysis indicated that parvalbumin (PV) and somatostatin (Sst) interneurons of the hippocampal CA1 subfield expressed Drd2 mRNAs. First, we examined the intrinsic excitability of D2R-expressing cells. Our data suggest that pharmacological D2R activation alters the intrinsic excitability of these hippocampal interneurons, by increasing the firing of action potential in Sst cells acting through the beta-arrestin pathway and decreasing the firing of PV cells by using the Gi/0 signaling pathway. These interneuron subtypes entrain pyramidal cells to orchestrate information processing and are instrumental in generating hippocampal oscillations. Using electrophysiological recordings of pyramidal cells, we observed that application of D2R-like agonist quinpirole (10 min, 10µM) in the presence of methacholine (muscarinic agonist, 50 nM) increases the number of theta oscillation periods by acting through the D2R on the Sst cells (Sst-D2R-Cko) but not via the PV cells (PV-D2R-Cko). These data show that activation of D2R of hippocampal interneurons modulates the activity of the hippocampal network. Ultimately this work should provide a better understanding of mnesic deficiency observed in dopaminergic transmission dysfunctions.

1-K-48 Characterizing the binding of bupropion and ibogaine to the dopamine transporter

Erin Williams[1], Matthieu Schapira[1], Ali Salahpour[1]
[1]University of Toronto

Individuals afflicted with Dopamine Transporter Deficiency Syndrome (DTDS) have minimal to no dopamine transporter (DAT) proteins at plasma membranes. Pharmacological chaperones selectively binding the DAT present a unique and viable path for treatment of select DTDS disease-causing variants. Two such compounds have been previously identified: bupropion and ibogaine, both atypical DAT inhibitors. However, it is not well understood how these drugs bind to the orthosteric binding site of DAT and mediate chaperone activity. Therefore, characterizing the binding sites of bupropion and ibogaine on DAT will provide critical information about the nature of the DAT orthosteric binding site and will aid in identifying more efficacious and potent pharmacological chaperones for treatment of DTDS. We used molecular modeling of the human DAT to investigate the potential binding site of bupropion and ibogaine to DAT. Currently, the predicted binding site and key residues involved in the binding of bupropion and ibogaine are being explored using site-directed mutagenesis followed by assessment of inhibition of binding and chaperone activity of the compounds. Our studies will provide key information as to which residues are necessary for bupropion and ibogaine binding and pharmacological chaperone activity.

1-K-49 Dynamic regulation of striatal acetylcholine release by D2-dopamine receptors and adaptations in a mouse model of Parkinson's disease

Stefania Vietti-Michelina[1], Emanuel Lopes[1], Katherine Brimblecombe[1], Stephanie Cragg[1]
[1]University of Oxford

Striatal dopamine (DA) axons and cholinergic interneurons (ChIs) exert strong reciprocal modulation on their activity and neurotransmitter release. Acetylcholine (ACh) exerts a range of effects on DA output, from directly driving axonal DA release bypassing action potentials in DA soma, to promoting short-term depression of DA release during train stimulation. In turn, DA can reciprocally inhibit firing activity from striatal ChIs predominantly via D2 DA receptors (D2Rs). Changes in D2R inhibition of ChIs in pathophysiological states such as Parkinson's disease (PD) or in the presence of addictive drugs can therefore be hypothesised to lead to changes in the dynamics of ACh release but until now, tools to understand ACh release dynamics have been limited. In ex vivo slices of mouse striatum we imaged the genetically encoded fluorescent sensor for ACh, GRABACh, to detect sub-second ACh regulation by D2Rs alongside detection of DA using fast-scan cyclic voltammetry, to identify D2R regulation of ACh by endogenous DA, and the impacts of cocaine or a modest deficit in DA release seen in a mouse model of early PD. We show that ACh release is under dynamic regulation by endogenous DA via D2Rs, with differences between dorsal and ventral striatum. We find that elevation of extracellular DA by cocaine partially inhibits ACh release via D2Rs, with pronounced regional differences. Conversely, we show that modest deficits in DA release in an α-synuclein-overexpressing model of early PD led to a maladaptive compensation in D2R regulation of ACh release. Overall, these findings reveal a strong and dynamic modulation of ACh by endogenous DA, its variation between striatal regions and dysregulation in different pathophysiological states that will shape ACh signalling.

1-K-50 Behaviorally penetrant, anomalous dopamine efflux exposes sex and circuit dependent regulation of dopamine transporters

Adele Stewart[1], Felix Mayer[2], Raajaram Gowrishankar[2], Steven Shatkhin[2], Samantha Stilley[2], Randy Blakely[2]

[1]University of Iowa, [2]Florida Atlantic University Brain Institute

The prevalence, age of onset, and clinical symptomology of neuropsychiatric disorders differs by sex. Although altered dopamine (DA) signaling is a feature of many disorders, sex-dependent mechanisms uniquely responsive to DA that drive sex-dependent behaviors remain unelucidated. Here, we utilize mice expressing the efflux-prone dopamine (DA) transporter (DAT) Val559 variant, identified in patients with the attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD), to investigate the physiological and behavioral impacts of anomalous DA efflux (ADE). In vivo, Val559 ADE induces activation of nigrostriatal D2-type DA autoreceptors (D2ARs) that magnifies nonvesicular DA release by elevating surface trafficking of ADE-prone DATs. Indeed, DAT Val559 mice exhibit sex-dependent alterations in threat aversion, social behavior, and memory. In a search for underlying mechanisms, we discovered that the ability of ADE to drive D2AR regulation of DAT is both sex and circuit-dependent, with dorsal striatum D2AR/DAT coupling evident only in males, whereas D2AR/DAT coupling in the ventral striatum is exclusive to females. As a result, compromised DA clearance was confined to mesolimbic and nigrostriatal DA projections in female and male DAT Val559 mice, respectively. Notably, systemic administration of the D2R antagonist sulpiride, which precludes ADE-driven DAT trafficking ex vivo, can rescue sex-biased memory deficits in DAT Val559 mice, but failed to normalize male-specific alterations in sociability and female-specific anxiety. Our work demonstrates that, rather than determining susceptibility to psychiatric illness, sex shapes the trajectory of shared biological insults resulting in categorically distinct physiological and behavioral changes in each sex.

M - Dopamine and behavior

1-M-51 Dopaminergic correlates of effort disutility in non-human primates

Mark Burrell[1], Alex Pastor-Bernier[2], Wolfram Schultz[2]
[1]Not listed, [2]University of Cambridge

Reward follows work, but with finite energy, all life must decide what reward is worth the work. Economic theory suggests how decision makers optimise this choice: agents should maximise the possible utility discounted by the disutility (subjective cost) of the required effort. The dopamine reward prediction error signal is a potential neuronal substrate of this calculation. Here, we test if the dopamine signal reflects net utility. Two rhesus macaques made binary choices in which reward and associated effort were varied. From these choices, we independently estimated utility of juice reward and disutility of effort on the same scale using random utility models, validating with out-of-sample tests. The midbrain dopamine neuron activity was measured using single-cell extracellular recordings. Empirical choice behaviour was closely matched to net utility predictions, providing evidence for the subtractive model of effort disutility. When accounting for changes in the disutility of effort, dopamine neuron activity reflected net utility at onset of stimuli. Furthermore, using stimuli indicating two possible effort levels, bidirectional effort prediction errors were elicited that scaled with effort disutility. By comparison to reward prediction errors elicited by risky reward cues, we demonstrate that dopamine neurons encode reward and effort on a common utility scale. These data suggest dopamine neurons capture the cost of effort by encoding net utility and hence convey value signals that are appropriate for decision-making involving effort.

1-M-52 Dopamine neurons reflect changes in internal subjective value estimates on a moment-to-moment basis

Daniel Hill[1], Robert Hickman[1], Arkadiusz Stasiak[1], Wolfram Schultz[1]
[1]University of Cambridge

Dopamine neurons play an essential role in reward valuation, signaling the subjective value (e.g., utility) of choice options. However, typical experimental methods to elicit subjective value suffer three key shortcomings: 1) subjective value is not directly reported but rather inferred from choices; 2) choice sets are often ordinal and preclude the study of value cardinality (i.e., probing 'whether' choice options differ rather than 'by how much'); and 3) hundreds of trials are required to infer choice probabilities, obscuring trial-to-trial changes in value. For these reasons, the fidelity of dopamine neuron encoding of subjective value on a trial-by-trial basis remains unclear. To more precisely understand how dopamine neurons encode subjective value from moment-to-moment, we trained rhesus monkeys to bid in a Becker-DeGroot-Marschak (BDM) auction task to elicit their willingness-to-pay for juice rewards while recording from midbrain dopamine neurons. The BDM is incentive compatible, meaning the optimal strategy is to bid one's true value; each bid offers an accurate trial-by-trial report of the monkey's internally generated subjective value that can be correlated with dopamine neuron activity. Neuronal responses to reward cues varied with subsequent animal bids even when reward magnitude was held constant. Although the fidelity of bid coding within single dopamine neurons was relatively low, support vector regression revealed that bids were accurately decoded when groups of neurons were analyzed, indicating that value estimates are constructed at a population level. These data show that dopamine responses reflect an intrinsic value estimate of goods prior to actions taken to acquire them, guiding behavior to obtain the highest subjective value for a given moment.

1-M-54 The small GTPase Rit2 modulates dopamine transmission

Mélie Talaron[1], Julia Obergasteiger[1], Anne-Marie Castonguay[1], Marina Ihidoype[1], Christophe Proulx[1], Mattia Volta[2], Martin Lévesque[1]
[1]Université Laval, [2]Eurac Research

The Rit2 gene was identified in recent genome wide association studies (GWAS) as a novel risk factor for Parkinson's disease (PD). Rit2 is a small GTPase only expressed in neural tissue, but its function has been scarcely investigated until now. Previous studies have shown that Rit2 can interact with the dopamine transporter and that Rit2 could regulate neurite outgrowth through the MAPK pathway. To reveal the role of Rit2 in the dopaminergic system, we performed both gain and loss of function experiments. Overexpression of Rit2 in mouse primary dopaminergic (DA) neurons promotes neurite outgrowth. Strikingly, overexpression of Rit2 in the substantia nigra (SNc) induces hyperlocomotion in mice in the open field test and a rotation phenotype in the cylinder test. We hypothesize that the hyperactivity depends on increased dopamine levels in the striatum and therefore, we are investigating the function of Rit2 in dopamine release and reuptake. To measure dopamine dynamics in freely moving mice, we are using fiber photometry combined with AAV-mediated expression of the dopamine sensor DLight in the striatum and Rit2 (or control vector) in the SNc of DAT-Ires-Cre mice. In parallel, we are using human iPSC-derived dopamine neurons and we are testing if Rit2 modulation affects dopamine dynamics in vitro and could have an impact on the network activity. This study is shedding light on the physiological functions of Rit2 in the dopaminergic system and can provide new insights on neurodegeneration mechanisms in PD.

1-M-55 A mosaic of dopamine dynamics: assessing the role of dopamine neuromodulation in habit learning

Oren Princz-Lebel[1], Miguel Skirzewski[1], Claire Lemieux[2], Daniel Palmer[1], Marco Prado[1], Vania Prado[1], Penny MacDonald[1], Lisa Saksida[1], Timothy Bussey[1]
[1]Western University, [2]McMaster University

Strategies for routine behaviours, or habits, provide a rapid, efficient means for decision making, but come with a loss of behavioural flexibility. Many psychiatric and neurodegenerative disorders are characterized by aberrant decision-making and dysfunctional habit formation, including addiction and obsessive-compulsive disorder. Striatal neurocircuitry underlies the habitual control of behaviour by facilitating synaptic plasticity and strengthening stimulus-response (S-R) associations. One essential neurotransmitter that regulates activity within the striatum is dopamine, and a loss of modulatory control of striatal dopamine has been shown to impact the rate of habit formation and associated processes. However, little is known about how S-R learning is supported by fast changes in extracellular dopamine levels across different striatal subregions. Here, we uniquely combined automated touchscreen cognitive assessments, fibre photometry, and the recently developed genetically-encoded dopamine biosensor, GRABDA, to record in vivo dopamine dynamics across the dorsomedial striatum, dorsolateral striatum and nucleus accumbens while mice performed the Visuomotor Conditional Learning Task- an established cognitive task that assesses S-R learning. We show that dopamine responds dynamically during the acquisition of S-R learning, and that these response patterns differ topographically across the striatum. Together, these findings suggest that the dopamine system in different striatal subregions plays distinct, but complementary, roles in stimulus-response learning.

1-M-56 Role of dopamine neurons in ultradian behavioral rhythms

Clément Bourguignon[1], Lei Zhu[2], Pratap Markam[1], Bruno Giros[3], Yulong Li[4], Kai-Florian Storch[3]
[1]McGill University / Douglas Institute, [2]Douglas Institute, [3]McGill University, [4]Peking University

It is well established that the daily pattern of activity and rest is shaped by the circadian clock and the light:dark cycle. There is evidence, however, that an additional system producing rhythms in the ultradian range contributes as well. Our previous findings suggest that dopaminergic neurons play a critical role in ultradian rhythm generation, possibly harboring the generator itself. Here we present evidence for ultradian fluctuations of cytosolic calcium in dopamine neurons based on intravital calcium indicator sensing. We show that this fluctuation is synchronous to ultradian locomotor rhythms in mice whose circadian clock function has been genetically disrupted. We further show that that these behavioral rhythms are also in synchrony with fluctuations of extracellular dopamine in the striatum as measured by a genetically encoded dopamine sensor. Together these data may help to delineate the mechanistic underpinnings of ultradian behavioral rhythms in 2-6hr range.

N - Other

1-N-57 Cardio-Metabolic and Psychiatric Comorbidities: Early Adversity- Mesocorticolimbic Dopamine Gene Network Interactions

Barbara Barth[1], Danusa Mar Arcego[1], Euclides De Mendonça Filho[2], Randriely Merscher Sobreira de Lima[2], Irina Pokhvisneva[3], Zihan Wang[1], Michael Meaney[1], Patricia Pelufo Silveira[1]
[1]McGill University, [2]Universidade Federal do Rio Grande do Sul, [3]Ludmere Centre

Psychiatric disorders are commonly comorbid with cardio-metabolic conditions through largely unknown biological pathways. We hypothesized that early life adversity would functionally link these conditions with the mesocorticolimbic dopamine system as a critical moderator pathway. We tested this hypothesis using a co-expression based polygenic score (ePRS) reflecting variations in the function of the dopamine transporter (DAT) gene network in the prefrontal cortex and striatum, the final targets of the mesocorticolimbic pathway. As predicted, the mesocorticolimbic DAT1 ePRS significantly moderated the impact of early life adversity on the risk for both psychiatric (schizophrenia, neuroticism, mood and substance use disorders) and cardio-metabolic (type 2 diabetes, atherosclerosis, cardiovascular disease) comorbidities in adults (UK Biobank, N= 60016) and adolescents (ALSPAC, N= 910). Brain gray matter densities in the insula and prefrontal cortex were significantly associated with SNPs from the DAT1 ePRS implicating these regions as critical dopaminergic targets for psychiatric/cardio-metabolic comorbidities. These results reveal that psychiatric and cardio-metabolic comorbidities share common developmental pathways and underlying biological mechanisms.

Poster Session 2

A – Dopamine, motivation, reward and addiction

2-A-1 Chronic administration of D2/3 agonist ropinirole enhances the ability of win-paired cues to drive development of long-lasting preference for risky choice in a rat gambling task

Leili Mortazavi[1], Tristan Hynes[2], Catharine Winstanley[2]
[1]Stanford University, [2]University of British Columbia

Dopamine replacement therapies (DRTs) such as ropinirole are common treatments for Parkinson's Disease (PD). While effective in mitigating the motor symptoms of PD, they can induce development of impulse control and gambling disorders in a considerable proportion of patients with chronic use. To investigate the mechanisms by which DRTs precipitate these conditions, we tested the effects of chronic administration of D2/3 receptor agonist ropinirole on animal models performing a rat Gambling Task (rGT). Male Long-Evans rats (N=112) received either saline, 2.5 or 5 mg/kg/day ropinirole via subcutaneously implanted osmotic pumps over 28 days while they acquired the rGT. In this task, animals choose between four options with varying probability and magnitude of winning sucrose pellets or losing time. Half the animals were trained on a cued version of the task where delivery of rewards was paired with audiovisual cues. We found that only in the animals performing the cued rGT, administration of ropinirole, specifically during the acquisition phase of the task, biased rats towards the high-risk/high-reward options. This risk preference remained and became progressively more pronounced long after termination of drug treatment. Furthermore, consistent with previous research on the rGT, motor impulsivity was dissociable from choice effects in that it only increased transiently but returned to normal levels before the end of the drug delivery period. These findings suggest a critical role for D2/3 activity to specifically modulate the ability of win-paired cues to increase preference for risky choice. Put together with previous work, this effect may be especially powerful when options are being sampled and evaluated but not after a preference has been shaped.

2-A-2 Methamphetamine-induced locomotor response varies depending on previous high or low sexual stimulation in male rats

Valeria Violante-Soria[1], Silvia Cruz[1], Gabriela Rodríguez-Manzo[1]
[1]Cinvestav

Sexual exhaustion in male rats consists of a long-lasting sexual inhibition (up to 72 h) that results from repeated ejaculation within a short period (around 2.5 h). This binge-like copulatory behavior is accompanied by a sustained increase in dopamine (DA) release in the nucleus accumbens (NAcc); however, 24 h later, DA levels decrease below initial basal levels. D-methamphetamine (Meth) dose-dependently increases locomotor activity due to the release of DA in the NAcc. High Meth doses produce stereotyped behaviors. We tested the hypothesis that Meth induces a different motor response in sexually exhausted males and in rats that ejaculated only once. We recorded locomotor activity in sexually experienced male Wistar rats that copulated to satiety (ad libitum copulation) or ejaculated once, 24 h prior to Meth (0.3, 1, 1.7, 3 mg/kg) or vehicle injection, along 90 min. The 1, 1.7 and 3 mg/kg Meth doses increased locomotor activity in animals of both sexual conditions; however, males ejaculating once achieved its maximal locomotor response after the 1.7 mg/kg dose and 3 mg/kg Meth induced stereotypies. By contrast, the sexually satiated males had their maximal response at 3 mg/kg Meth without showing stereotypies. In conclusion, satiated rats with reduced NAcc DA levels required a higher Meth dose to achieve its maximal locomotor response and were less susceptible to exhibit stereotypies as compared to males that ejaculated once, suggesting that the sexual history influences the magnitude of the motor responses (motor activity and stereotypies) induced by a high Meth dose.

2-A-4 Developmental experience of food insecurity affects adult responses to negative outcomes and uncertainty and dopamine neurobiology

Wan Chen Lin[1], Christine Liu[1], Polina Kosillo[1], Lung-Hao Tai[1], Ezequiel Galarce[2], Helen Bateup[3], Stephan Lammel[4], Linda Wilbrecht[1]

[1]University of California, Berkeley, [2]Optum Labs, [3]University of California Berkeley, [4]University of Minnesota

Food insecurity, scarce and uncertain access to food, is influencing over millions of households with children and adolescents worldwide. Currently, it is not fully clear if transient food insecurity experience during the juvenile-adolescent developmental period affects adult learning, cognitive development, and dopamine system. We developed a mouse model of food insecurity, manipulating feeding schedules only during the juvenile-adolescent period from postnatal day(P)21 to 40 as food insecure (FI) or ad libitum (AL). We found that adult males in the FI and AL groups showed significantly different dopamine neurobiology and behavioral profiles. In separate cohorts of adult male mice, we examined the synaptic plasticity of dopamine neurons in the ventral tegmental area (VTA) and evoked striatal dopamine release in ex vivo slices. We found that AMPAR/NMDAR ratio in the nucleus accumbens (NAc) core-projecting VTA dopamine neurons and dopamine release in the dorsal striatum were significantly decreased in the FI group compared with the AL group. Behaviorally, we found that adult male FI and AL mice showed significantly different performance in the reversal phase of a deterministic 4-choice odor-based foraging task and in a probabilistic 2-armed bandit task when probability of reward was 65-75% but not 90%. Applying reinforcement learning models to behavioral data, we found that juvenile-adolescent (P21-40) feeding experience affected sensitivity to negative outcomes in both tasks, but the direction of effect was task specific. Together, these data show in a rodent model that transient differences in food scarcity and uncertainty in development can have lasting effects on learning, decision-making, and dopamine function in adulthood.

2-A-5 Midbrain dopamine is sensitive to Pavlovian information loss

Eric Garr[1], Aneesh Bal[1], Yifeng Cheng[1], Laia Castell[1], Sara Brooke[1], Patricia Janak[1]
[1]Johns Hopkins University

There has long been evidence that Pavlovian learning is a function of the mutual information between cue and reward--the degree to which a cue signals a change in reward rate. We investigated whether midbrain dopamine dynamics are sensitive to Pavlovian information loss. We used fiber photometry to record from dopamine neurons in the ventral tegmental area (VTA) during cue-reward conditioning followed by contingency degradation in TH-Cre+ rats (n = 13). Contingency degradation attenuated the rate, timing, and latency of conditioned port entries. Calcium transients at the time of reward were sensitive to local reward history in a manner consistent with prediction error encoding, but this sensitivity disappeared with contingency degradation. Calcium transients at the time of cue onset did not depend on local reward history regardless of cue-reward contingency, opposing the prediction error hypothesis. We further show that sensitivity to contingency degradation is predicted by how much larger the dopamine response is to non-contingent rewards. In a separate experiment, we asked whether degrading the contingency between a cue and optogenetic VTA dopamine neuron stimulation would attenuate conditioned locomotion. During acquisition, conditioned locomotion increased in TH-Cre+ rats (n = 16), but not in TH-Cre- rats (n = 7) or TH-Cre+ rats that experienced contingency degradation (n = 6). Rats that acquired the conditioned response were split into groups that were either maintained on the conditioning protocol or underwent contingency degradation. Conditioned locomotion was attenuated only in the latter group. Together, these results suggest that phasic VTA dopamine neuron activity outside of conditioning trials can support behavioral sensitivity to contingency degradation.

2-A-6 Simultaneous genetic access to the brain's dopamine and serotonin systems reveals neuromodulatory dynamics underlying Pavlovian learning

Daniel Cardozo Pinto[1], Matthew Pomrenze[1], Jayashri Viswanathan[1], Brandon Bentzley[1], Neir Eshel[1], Robert Malenka[1]
[1]Stanford University

Survival depends on an organism's ability to seek rewards and learn about environmental cues that predict them. Two of the brain's major neuromodulatory systems - dopamine (DA) and serotonin (5-hydroxytryptamine; 5HT) - are thought to play a critical role in this Pavlovian (i.e. cue-outcome) learning process because they respond to rewards and project to downstream targets involved in shaping motivated behaviors. However, the neural mechanisms underlying the integration of convergent neuromodulatory teaching signals to drive learning remain poorly understood, in large part because it has been technically difficult or impossible to study multiple neurotransmitter systems at once. Here we report the development of DAT-Cre/SERT-Flp mice; a double transgenic model enabling simultaneous, independent genetic access to the dopamine and serotonin systems in a single animal. Anterograde axon tracing in these mice revealed that ventral tegmental area (VTA) DA and dorsal raphe (DR) 5HT neurons innervate limbic targets with region and subregion specificity. Then, focusing on the structure with the greatest density of converging modulatory inputs - the nucleus accumbens (NAc) - we applied two color fiber photometry to simultaneously record the activity of DA and 5HT axons during Pavlovian learning. We find that DA axons are excited during reward consumption while 5HT axons are inhibited. Finally, we show that DA receptor 1 (D1) expressing medium spiny neurons (MSNs) in the NAc preferentially express inhibitory 5HT receptors, while DA receptor 2 (D2) expressing MSNs preferentially express excitatory 5HT receptors. Altogether our findings suggest that DA and 5HT signals converging in the NAc may synergistically excite D1 MSNs and inhibit D2 MSNs to drive Pavlovian reward learning.

2-A-7 VTA circuitry sustains opposite responses of dopaminergic neurons to drugs of abuse

Tinaïg Le Borgne[1], Claire Nguyen[1], Eleonore Vicq[1], Stefania Tolu[1], Alexandre Mourot[1], Fabio Marti[1], Philippe Faure[1]
[1]CNRS - ESPCI

AIMS: Nicotine, the main psychoactive compound of tobacco, binds on cationic nicotinic receptors (nAChRs) to increase the firing rate of dopaminergic (DA) neurons of the ventral tegmental area (VTA), leading to higher release of DA in target structures and positive reinforcement. Besides rewarding properties, nicotine also promotes negative effects. Recently, we showed heterogeneity in nicotine-induced responses on VTA DA subpopulations targeting nucleus accumbens (NAc) and amygdala (AMg) nuclei. NAc-projecting DA neurons are activated by nicotine and their optogenetic activation is reinforcing. AMg-projecting DA neurons are inhibited by nicotine and their optogenetic silencing is anxiogenic. We address 1) if alcohol, known for its rewarding and anxiolytic/anxiogenic properties, also produces distinct responses on DA subpopulations, and 2) if a circuit-based mechanism could underly drug-induced inhibition. METHODS: Combining in vivo juxtacellular or ex vivo patch-clamp recordings with injections of retrograde tracers, we investigated extrinsic/intrinsic properties and response profiles to nicotine and ethanol of NAc or AMg-projecting DA neurons. RESULTS: We demonstrated that alcohol induces two opposite responses, such as nicotine, on the same segregated DA subpopulations. NAc-projecting DA neurons are activated by nicotine and ethanol while AMg-projecting DA neurons are inhibited by both drugs. Finally, we start to investigate intrinsic properties, difference in receptor expression and local/distal GABAergic signaling of both populations. CONCLUSION: These results

highlight heterogeneity of drug impact on DA subpopulations and raise the question of the role it might play in the balance between the drugs positive and negative effects that regulates their use

2-A-8 Self-administration of right vagus nerve stimulation is modulated by satiety

Ching-Tzu Tseng[1], Hailey Welch[1], Catherine Thorn[1]
[1]The University of Texas at Dallas

The vagus nerves are important carriers of sensory information from the viscera to the CNS, and are key in supporting the food-seeking behavior that is necessary for survival. Vagus nerve signaling has been shown to reinforce learned behaviors via activation of midbrain "reward" circuits, including the VTA and SNc. Recent work from our lab demonstrates strong lateralization of this vagal reward-related signaling, and suggest that r-VNS may offer a therapeutic strategy for activation of the midbrain DA system, which has broad implications for the treatment of mood and motor disorders. Additional research is needed, however, to determine the translational potential of r-VNS driven DA signaling. As a first step, we tested whether r-VNS self-administration (SA) is sensitive to hunger/satiety, as metabolic state is well known to modulate vagus-mediated reward seeking in other contexts. Adult Long Evans rats were implanted with r-VNS electrodes 1 week prior to study start. After recovery, rats were placed on restricted food access and received 1-3 lever-habituation sessions in which a food pellet was received for each correct lever press. After habituation, food pellets were removed and 5 VNS SA sessions were performed (days 1-5), in which each press was paired with the presentation of a visual cue and r-VNS delivery. After SA session 5, rats were switched to ad libitum access to food in their home cages and VNS SA training continued for another 5 sessions (days 6-10). After session 10, food access was again restricted, and rats received a final 5 days of VNS SA. Preliminary results indicate that VNS reinforced lever pressing is reduced during periods of free access to food, consistent with strong modulation of vagal-midbrain signaling by satiety state.

2-A-9 Mesocorticolimbic function in recreational cocaine users: a multimodal study of altered cue reactivity and cognitive regulation

Stephanie Scala[1], Min Su Kang[2], Sylvia M.L. Cox[1], Marco Leyton[1]
[1]McGill University, [2]Sunnybrook Research Institute

Extended cocaine use leads to decreased dopamine and glutamate receptor levels resulting in impaired learning and cognitive control including poorly regulated responses to drug-related cues. Our lab previously reported that dopamine D2 receptor (DRD2) availability was not decreased in recreational stimulant users. In the present study, we characterized mesocorticolimbic network function and the availability of type 5 metabotropic glutamate receptors (mGluR5s). Fifteen recreational cocaine users (RCUs) and 15 healthy controls (HCs) completed an fMRI scan while viewing cocaine and neutral videos. To examine cognitive control circuit function, participants were instructed to regulate their desire for cocaine for half of the cocaine videos by thinking of negative consequences of cocaine use. Most participants (15 RCUs, 14 HCs) also completed a PET scan with the tracer [11C]ABP688, which binds to mGluR5. Compared to neutral cues, RCUs showed drug cue reactivity in areas such as the striatum, thalamus, and midbrain. During regulation (vs. non-regulation) trials, RCUs showed reduced drug cue activation in the anterior cingulate, ventromedial prefrontal cortex, and ventral striatum. [11C]ABP688 binding was similar for HCs and RCUs with limited cocaine experience (15-50 uses), but reduced in those who had used the drug more often (>75 uses). The lower the prefrontal [11C]ABP688 binding, the greater the activations during regulation trials in the hippocampus, amygdala, and midbrain. Together,

these findings suggest that (i) RCUs exhibit mesocorticolimbic network responses to cocaine cues, and (ii) impairments in mGluR5 availability appear before decreases in DRD2. These reductions in mGluR5 might diminish the ability to regulate cue-induced mesocorticolimbic reactivity.

2-A-10 Dopamine release increases when sensory cues are presented with rewards in risky gambles

Lester Tong[1], Connor Bevington[1], Mariya Cherkasova[2], Ju-Chieh Kevin Cheng[1], Jordan Hanania[1], A Jon Stoessl[1], Vesna Sossi[1], Luke Clark[1], Catharine Winstanley[1]
[1]University of British Columbia, [2]West Virginia University

D3 receptor binding mediates increased risk seeking in a rat gambling task when presented with salient win-related audiovisual cues. We explored whether audiovisual cues in the Vancouver gambling task (VGT; a two-choice lottery task) elicited increased dopaminergic activity relative to reward outcomes without added cues. Five healthy community members chose between pairs of risky gambles in the VGT while undergoing [11C] raclopride positron emission tomography combined with functional magnetic resonance imaging (PET-FMRI). The protocol began with bolus and infusion administered 36 min prior to the first task block (~14 min), followed by a 10 min break, the second task block (~14 min), and ended when 90 min elapsed. Audiovisual cues were presented with win outcomes in the "cued" block of the VGT, but not in the "uncued" block. Block order was counterbalanced across participants. We modeled the time-varying raclopride binding potential to estimate dopamine release magnitude across each task. Overall, the magnitude parameter was greater in cued compared to uncued blocks ($t=2.92$, $p=0.04$). Subjects that saw the cued (M=5645) block first had comparable dopamine release magnitude to those that saw the uncued (M=5099) block first. However, seeing the cued block after the uncued block elicited additional dopamine release (M=7371), while a reduction in magnitude was observed in subjects viewing the uncued block after the cued block (M=3164). Pairing wins with audiovisual cues therefore does seem to enhance dopamine release, perhaps indicative of greater incentive salience. Future research will test associations between current PET results and FMRI data, with the aim of comparing problem gamblers and healthy controls to describe the neurobiological basis of gambling disorder.

2-A-11 Chemogenetic manipulation of the dopaminergic nigrostriatal pathway alters the development and maintenance of cue-induced risky choice in female rats

Brett Hathaway[1], Andrew Li[1], Hannah Brodie[1], Mason Silveira[1], Melanie Tremblay[1], Catharine Winstanley[1]
[1]University of British Columbia

Deficits in cost/benefit decision making are a critical risk factor for addiction. Reward-concurrent cues may mediate this relationship, as such stimuli enhance risk preference and potentiate cocaine intake in rats. Despite extensive research implicating the dorsal striatum in compulsive drug seeking, the role of dorsal striatal dopaminergic activity in cue-induced risk preference remains unclear. Accordingly, we examined the effects of manipulating the dopaminergic nigrostriatal pathway on cue-induced risky decision making in rats. Female TH:Cre rats were trained on the cued version of the rat Gambling Task (rGT). This task was designed such that maximal reward is attained by avoiding the high-risk, high-reward options and instead favouring the options associated with lower per-trial gains, as they feature less frequent and shorter time-out penalties. Adding reward-paired audiovisual cues to the task leads to greater risky choice on average. To assess the role of the nigrostriatal pathway, Cre-dependent inhibitory or excitatory DREADD was infused into the substantia nigra. Rats then received Clozapine-N-Oxide either during the learning phase (first 30 sessions) or after a stable performance baseline was

reached. Activation of the pathway during task learning delayed the establishment of a risky decision-making profile in risk-preferring rats, and reduced risky choice in all rats when baseline performance was targeted. Conversely, inhibition of this pathway accelerated the development of risk preference, and increased risky choice during performance. These results provide evidence for the involvement of the dopaminergic nigrostriatal pathway in cue-induced risk preference, therefore shedding light on its role in cost/benefit decision-making deficits in humans.

2-A-12 Adolescent Social Isolation Drives Increased Heroin Vulnerability through Dysregulation of the Dopamine System

Brianna George[1], Sara Jones[1]
[1]Wake Forest School of Medicine

Our group has previously found that adolescent social isolation (aSI) stress in rats leads to increased negative affective behaviors, alterations in dopamine system functioning, and cocaine and alcohol consumption in adulthood compared to group-housed (aGH) controls. Here, we explore the impact of aSI on heroin SA and heroin-induced dopamine alterations. Our results revealed that aSI rats have significantly increased rates of acquisition, breakpoints on progressive ratio, and escalation of responding on long access in adulthood. Using FSCV, we found that stimulated dopamine release in the nucleus accumbens (NAc) was reduced in both aSI and aGH heroin-exposed rats when compared to naïve counterparts, however; uptake rates were only reduced in heroin-exposed aSI rats. We also found that heroin aSI rats had increased activity at D2/D3 autoreceptors in the NAc, suggesting greater downregulation of the dopamine system in heroin-exposed aSI rats. Further, we found increased 22 kHz USVs in heroin aSI rats, suggesting greater negative-affect in heroin-exposed aSI rats during withdrawal. In sum, we demonstrate that aSI results in robust behavioral and neurobiological adaptions that increase vulnerability to opioids. Additionally, the intersection of aSI and heroin vulnerability may be linked to altered dopaminergic functioning in reward-related brain regions.

2-A-13 Serotonin2B receptor antagonists potentiate cocaine-induced dopamine release in the medial prefrontal cortex: implication in the control of cocaine-induced hyperlocomotion

Adeline Cathala[1], Jean Michel Revest[1], Umberto Spampinato[1]
[1]University of Bordeaux

The central serotonin2B receptor (5-HT2BR) is currently considered as a potential pharmacological target for treating cocaine addiction. Indeed, 5-HT2BR antagonists have been shown to inhibit cocaine-induced hyperlocomotion, a behavioral response classically assessed to predict the reinforcing properties of drugs of abuse. This suppressive effect occurs independently of subcortical dopamine (DA) outflow, and could involve a direct post-synaptic modulation of subcortical DA transmission. In this context, medial prefrontal cortex (mPFC) DA could play a key role, as it is known to inhibit subcortical DA transmission and DA-dependent behaviors. However, the impact of 5-HT2BRs in the control of cocaine-induced DA outflow in the mPFC is unknown to date. The present study, combining intracerebral microdialysis in freely moving rats and behavioral assessments, investigated the effect of 5-HT2BR antagonists on cocaine-induced DA outflow in the mPFC, and the role of mPFC DA in their suppressant effect on cocaine-induced hyperlocomotion. We found that 5-HT2BR antagonists, which per se increased basal DA outflow, potentiated cocaine-induced DA outflow in the mPFC. Also, 5-HT2BR antagonists reduced cocaine-induced hyperlocomotion, this suppressant effect being no longer observed in animals bearing the lesion of the mesocortical DA pathway. These findings suggest that 5-

HT2BR antagonists by potentiating cocaine-induced DA release in the mPFC could trigger changes of subcortical DA transmission, resulting in the suppression of cocaine-induced hyperlocomotion. Overall, this study affords additional knowledge on the regulatory control exerted by the 5-HT2BR on ascending DA pathways and on cocaine-induced neurochemical and behavioral effects.

2-A-14 Striatal dopaminergic gene network interacts with exposure to early adversity influencing food intake and emotional regulation in a developmental trajectory

Kelly Scudine[1], Randriely Merscher Sobreira de Lima[2], Barbara Barth[3], Irina Pokhvisneva[4], Danusa Mar Arcego[3], Zihan Wang[3], Carla Dalmaz[2], Michael Meaney[3], Paula Midori Castelo[5], Patricia Silveira[3]
[1]Federal University of São Paulo (UNIFESP), [2]Universidade federal do rio grande do sul, [3]McGill University, [4]Ludmere centre, [5]Universidade Federal de São Paulo (UNIFESP)

Early life adversities affect food consumption and increase the susceptibility to diseases later in life through mechanisms not fully understood. We created here an innovative expression-based polygenic risk score to investigate the hypothesis that a dopaminergic co-expressed gene network (DRD4-ePRS) in striatum moderates the effects of early adversity on feeding and emotional development. The polygenic score significantly moderated the association between early life adversity on food/ liquid intake and emotional symptoms in 4 years old children from two samples (MAVAN N=138 and GUSTO N=436), and food intake and broad depression phenotype in a large adult cohort (UK Biobank, N=9,153). Anatomo-functional correlations were observed between variations in our polygenic score and brain gray matter density in cortical regions in both children and adults. In sum, our data suggest that a DRD4 co-expressed network in the striatum acts as a moderator of early life stress' influence on dietary and emotional behaviors from a developmental perspective.

2-A-15 Value vs prediction error: the role of vta da transients in associative learning

Alexandra Usypchuk[1], Etienne Maes[2], Megan Lozzi[1], Matthew Gardner[1], Guillem Esber[3], Mihaela Iordanova[1]
[1]Concordia University, [2]McGill University, [3]Brooklyn College

Phasic firing of ventral tegmental area (VTA) dopamine (DA) neurons is recognized as a prediction error signal that supports learning about reward. Whether the signal drives learning through an error mechanism or by regulating value is hotly debated due to the difficulty in isolating changes in value from prediction error. We addressed this long-standing debate using optogenetic activation of VTA DA neurons in a series of blocking experiments, which isolate the role of prediction error in learning. Each experiment consisted of a conditioning phase followed by a blocking phase and a manipulation-free test. In the first experiment, we tested whether activation of DA neurons at the time of reward delivery during blocking reinstates learning about the normally blocked cue. We found that learning is unblocked to the level of a non-blocking control cue. To determine whether activation of DA neurons unblocks learning by boosting the value of rewards or by directly encoding error, we delivered identical stimulation across the two training phases (i.e., conditioning and blocking). If boosting the VTA DA signal unblocks learning by increasing prediction error through increases in value, then we should find blocking. If the signal unblocks learning by acting on prediction error per se, then we should find unblocking. We optogenetically boosted the VTA DA signal at time of reward delivery across both phases. We found that learning was unblocked to the level of a control stimulus. These data reconcile that DA firing in the VTA functions directly to encode error.

D – Dopamine, Parkinson's Disease and neurodegeneration

2-D-16 Oxidation of parkin confers redox homeostasis in adult human brain

Michael Schlossmacher[1], Jacqueline Tokarew[1], Daniel El-Kodsi[1], Nathalie Lengacher[1], Travis Fehr[1], Juan Li[1], Quibo Jiang[1], Angela Ngyuen[1], Gergely Toth[2], Lawrence Puente[1], Peggy Taylor[3], Julianna Tomlinson[1]
[1]University of Ottawa, [2]Gardedam Therapeutics, [3]BioLegend Inc

Parkin deficiency leads to young-onset Parkinson disease (PD). We postulated that a key function of Parkin is to neutralize radicals via thiol chemistry. We tested this hypothesis by analyzing >50 human brains, recombinant proteins and dopaminergic cells. We found that after age 40 years, >90% of Parkin is normally found in SDS-soluble aggregates. This translocation from the cytosol correlates with age and Parkin's own oxidation, such as at cysteine residues 95 and 253. In vitro, the solubility of Parkin is progressively diminished by dopamine metabolites and hydrogen peroxide (H2O2). In the absence of Parkin, H202 levels are elevated. Parkin also conjugates dopamine metabolites, such as at cysteines 332 and 337, rendering it irreversibly oxidized. Wild-type but not PD-linked mutant Parkin protects human cells from dopamine-mediated toxicity, thereby forming SDS-soluble aggregates. In midbrain sections from neurologically healthy adults, newly developed, monoclonal antibodies to Parkin reveal its co-localization with phagosomal LC3B and lysosomal LAMP-3/CD63 within dopamine neurons. We conclude that in human brain Parkin normally undergoes reversible and irreversible modifications during normal ageing, which may confer protection from reactive radicals that promote oxidative stress.

2-D-17 Identification and validation of new therapeutic targets against Parkinson's disease by CRISPR-Cas9 screening at the genome level

Axelle Dovonou[1], Yves De Koninck[1], Emmanouil Metzakopian[2], Martin Lévesque[3]
[1]CERVO Brain Research Center, Laval University, [2]University of Cambridge, [3]Université Laval

Parkinson's disease (PD) is one of the most common neurodegenerative disorders affecting around 1% of the population aged 60 and over. It is characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra par compacta (SNc) causing the majority of motor PD symptoms. It is now well accepted that oxidative stress, mitochondrial defects or the toxicity of protein aggregate, are associated with the DA neuron degeneration. However, the mechanisms underlying this neuronal death remain unclear. There is no cure for PD and current treatments only alleviate symptoms of the disease. It is therefore essential to discover new therapeutic approaches for this devastating disease. The aim of this study is to identify new therapeutic targets against PD. To address this question, we carried out a genome-wide screening by applying a CRISPR-Cas9 library to identify target genes rescuing degeneration of DA neurons induced by the neurotoxin rotenone. Results from this non-bias screening revealed several candidate target genes that could potentially protect against oxidative stress and mitochondrial dysfunction. To validate these data in vivo, we generated mice expressing Cas9 in DA neurons and injected an adenoviral vector encoding specific guides RNAs directed against one of our previously identified target gene. In agreement with our in-vitro data, gene inactivation in vivo could protect dopamine neurons from parkinsonian phenotype induced by the neurotoxin 6-hydroxydopamine (6-OHDA). Our study identified new targets that confer neuroprotection both in vitro and in vivo models of PD. Results from this study could lead to new therapeutic treatments against neurodegeneration in PD.

2-D-18 Altered dopamine and glutamate synaptic transmission in the VPS35 (D620N) knock-in mouse model of Parkinson's disease

Anusha Kamesh[1], Austen Milnerwood[1]
[1]McGill University

Parkinson's Disease (PD) is a complex syndrome of motor and non-motor symptoms, resulting from the dysfunction of multiple neurotransmitter systems. Etiologically, genetic predisposition, in addition to environmental factors, is thought to lead to PD onset (Volta et al., 2015, Obeso et al., 2017). To address genetic predisposition, we compared the physiological and neurobiological alterations produced by two late-onset PD mutations in leucine-rich repeat kinase (LRRK2) and vacuolar protein sorting 35 (VPS35) proteins, in two distinct knock-in mouse models (Volta 2017., Beccano-Kelly et al., 2014; Cataldi et al., 2018). We have found both proteins have synaptic functions (Beccano-Kelly, Tatarnoikov et al 2014, Volta et al., 2015, Munsie, Milnerwood et al., 2014, Cataldi 2018), and that LRRK2 and VPS35 PD-linked mutations alter neurotransmitter release in the striatum. We observed increased dopamine release by fast scan voltammetry in both LRRK2 and VPS35 knock-in mice (Volta et al., 2017; Cataldi et al., 2018) as well as altered glutamatergic transmission in LRRK2 knock-ins (Volta et al., 2017). Glutamate can indirectly affect dopamine release through activation of striatal cholinergic interneurons (Kosillo et al., 2016; Threlfell et al., 2012). Here, we report alterations to glutamatergic activity in brain slices from VPS35 knock-in mice. We also describe the use of a dopamine biosensor (Patriarchi et al., 2018) and fast-scan cyclic voltammetry to further assay dopamine release in brain slices. By understanding the changes to neurotransmission preceding dopamine cell loss, we hope to identify a therapeutic window whereby intervention can provide neuroprotection, and ideally prevent disease onset altogether.

2-D-19 Regulation of L-type Ca2+ channels by alpha-synuclein

Se Joon Choi[1], Manu Ben-Johny[1], Mahalakshmi Somayaji[1], Ellen Kanter[1], David Sulzer[1], Eugene Mosharov[1]
[1]Columbia University

α-Synuclein (α-Syn) plays a central role in sporadic and familial Parkinson's Disease (PD) via a toxic gain-of-function mechanism, and a variety of experimental strategies are under development to decrease α-Syn levels in PD patients. α-Syn deficiency has been shown to alter the kinetics of dopamine release, but there has been relatively little analysis of changes in endogenous physiological responses in neurons expressing normal levels of the protein. We studied the effects of α-Syn deficiency on intrinsic electrophysiological properties in primary mouse neuronal cultures. Although substantia nigra (SN) dopaminergic neurons from α-Syn deficient cells showed overall normal electrophysiological properties, they had significantly lower L-type Ca2+ channels (LTCC) activity than cells from wild-type animals. This effect was likely related to a decrease in surface expression of LTCCs with no alteration in mRNA or single channel open probability and conductance. The decreased activity-dependent Ca2+ flux resulted in deficient pCREB induction - a central step in the regulation of immediate early genes expression required for synaptic plasticity. We conclude that strategies for α-Syn reduction should examine possible changes in LTCC activity and downstream second messenger systems that regulate synaptic plasticity and learning.

2-D-20 Intrinsic Alterations of Dopaminergic Neuron Physiology and Morphology in the 3xTg-AD Mouse Model

Harris Blankenship[1], Alex Lin[1], Sarah Ocanas[1], Kylie Handa[2], Michael Beckstead[2]
[1]University of Oklahoma Health Sciences Center/Oklahoma Medical Research Foundation, [2]Oklahoma Medical Research Foundation

Amyloid-β and tau accumulation accompanied by cortico-hippocampal degeneration comprise the pathological hallmarks of late-stage Alzheimer's disease, but do not readily explain all behavioral phenotypes present in Alzheimer's patients. Therapeutics designed to clear the brain of these protein aggregates has not yielded disease-modifying results, indicating that a better understanding of early pathophysiological mechanisms is required. Neuropsychiatric symptoms including apathy often develop prior to cognitive decline, suggesting dopaminergic system dysregulation. Here we used acute slice patch-clamp electrophysiology, RNA-sequencing, and morphological reconstruction to assess single dopaminergic neuron structure and function in the 3xTg-AD mouse model, which expresses both amyloid-β and tau in a characterized spatiotemporal manner. Recordings from 3xTg-AD mice indicated increased sensitivity to current injection by eighteen months of age. This may be explained by a decrease in small conductance calcium gated potassium channel (SK) currents, which declined prior to dysregulated action potential generation. We also observed an increase in the slow decay A-type potassium current, which may act as a compensatory mechanism to slow firing rates. Additionally, we observed diminished neurite branching at 20 months of age in the 3xTg-AD mice. We have also paired patch-clamp electrophysiology with single cell RNA sequencing and have determined that cells with similar physiology have similar gene expression, and that individual genes are correlated to physiological measures. These results suggest structural and functional alterations in dopaminergic neurons develop across the lifespan of 3xTg-AD mice and may point towards future therapeutic targets in Alzheimer's disease.

2-D-21 Function and neuroprotective potential of Flcn knockout in Parkinsons disease

Julia Obergasteiger[1], Thomas Durcan[2], Emmanouil Metzakopian[3], Martin Levesque[4]
[1]University of Cambridge, [2]McGill University, [3]UK Dementia Research Institute, [4]Université Laval

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by various motor and non-motor symptoms and by the loss of dopaminergic (DA) neurons. There is no cure for PD and current treatments only alleviate the symptoms of the disease. We performed a CRISPR-based, genome-wide screen to identify new target genes rescuing degeneration of DA neurons. From our screen we identified the Flcn gene. Flcn is implicated in the mTOR pathway, able to regulate autophagy and to interact with several Rab GTPases, involved in endocytic trafficking. To understand the physiological role of Flcn in DA neurons and validate its potential neuroprotective effect, we are performing a Flcn knockout (KO) in mouse dopamine neurons. For modelling PD, we used AAV-mediated expression of human alpha-synuclein (aSyn) in the mouse substantia nigra. Locomotor assessment in mice indicated that knocking out Flcn ameliorates the motor deficits induced by aSyn overexpression. Furthermore, Flcn-KO rescues the loss of dopaminergic neurons in the midbrain and their terminals in the striatum. In vitro, we used human iPSC-derived DA neurons with PD-related mutations and isogenic control lines and mouse primary neurons. We found that Flcn KO improves mitochondrial deficits observed in PD conditions. Here, we used an unbiased screening method to identify new neuroprotective targets for PD and following target validation, we will try to identify drugs capable of modulating Flcn and test their efficacy to modify disease onset and/or progression.

2-D-22 Investigating blood brain barrier damage and immune cell entry after Citrobacter rodentium infection in Pink1 knockout mice

Sriparna Mukherjee[1], Vladimir Grouza[2], Marius Tuznik[2], Amandine Even[1], Sherilyn Recinto[2], Marie-Joseé Bourque[3], Pedro Rosa-Neto[2], Jo Anne Stratton[2], Heidi McBride[2], Samantha Gruenheid[2], David Rudko[2],

Louis-Eric Trudeau[3]
[1]University of Montreal, [2]McGill University, [3]Université de Montréal

Research in the past decade established a strong link between immune system activation and the development of Parkinson's disease (PD). In a recent study, we showed that repeated gastrointestinal infection with Citrobacter rodentium can lead to PD-like symptoms in Pink1 KO mice. Here, we aimed to test the hypothesis that infection in this model leads to increased blood brain barrier (BBB) permeability, facilitating entry of immune cells in the brain, as well as chronic microglial activation. Pink1 WT and KO mice were infected with Citrobacter rodentium and at days 13 and 26 post infection, we conducted gadolinium-enhanced MR imaging to identify signs of BBB permeability. We also quantified expression of endothelial tight junction proteins and dopamine metabolites along with investigating systemic inflammatory mediators. Our MRI data provide evidence for little if any increase in gadolinium entry in the brain of Pink1 KO mice at both time points, as compared to uninfected mice. Small changes in systemic inflammation were also observed. However, signs of chronic microglial activation were detected at day 26 post infection. These results support the hypothesis that increased immune cell entry in the brain after gastro-intestinal infection does not result from a long-lasting increase in BBB permeability. Further studies are required to examine the links between immune cell entry in the brain of these mice and the appearance of dopamine neuron dysfunction. Our observation of increased microglial activation suggests that a chronic state of brain inflammation could also mediate some of the dysfunctions observed in these mice.

2-D-23 Characterization of a novel mouse model of Dopamine Transporter Deficiency Syndrome

Emma Russo[1], Ameneh Rezayof[2], Pieter Beerepoot[1], Amy Ramsey[1], Ali Salahpour[1]
[1]University of Toronto, [2]University of Tehran

Genetic mutations that result in misfolded proteins are a cause of a number of human diseases, one of which is Dopamine Transporter Deficiency Syndrome (DTDS). Autosomal recessive loss of function mutations in the dopamine transporter (DAT) lead to its retention and degradation in the endoplasmic reticulum (ER), and consequential low levels of functional DAT at the cell surface. A complex motor disorder arises, and patients diagnosed with DTDS display a classical clinical phenotype of initial hyperactivity that progresses over time to parkinsonism-dystonia. To gain additional information on the pathophysiology of this disease and to investigate potential pharmacotherapies we have created a new line of transgenic knock-in mice carrying one particular DTDS disease-causing DAT mutation. This mouse (mA313V) models the human DTDS mutation hA314V. Herein we report on the initial behavioral and neurochemical characterization of the A313V mice. A313V mice have approximately 5-fold less mature DAT in comparison to wildtype (WT) littermates and a consequent increase in locomotor activity as measured by the open field test. This hyperactivity can be reduced upon treatment of amphetamine (AMPH) and alpha-methyl-para-tyrosine (αMPT), suggesting that promising drug treatments for the management of classic DTDS symptoms exist. Our study characterizes a mouse model of DTDS and provides guidance for the management of DTDS symptoms via pharmacotherapy.

2-D-24 Knockdown of PlexinC1 in human induced pluripotent stem cells for efficient cell replacement therapy in Parkinson's disease

Tiago Cardoso[1], Martin Lévesque[2]
[1]Faculty of Medicine, Laval University, [2]Université Laval

Cell replacement therapy, where lost dopamine (DA) neurons in substantia nigra pars compacta are replaced by a source of healthy neurons, is a promising therapeutic avenue for Parkinson's disease (PD). Pre-clinical evidence shows that transplanted human pluripotent stem cell (hPSC)-derived dopamine (DA) neurons survive, innervate the host striatum and revert motor asymmetries in preclinical models of PD. However, only a fraction of the graft-derived DA axons reaches the functionally relevant dorsal striatum when placed in the midbrain. We have recently shown that the Sema7a-PlexinC1 guidance pathway controls axon innervation of DA neurons, in which SNpc neurons expressing low levels of PlexinC1 innervate the dorsal part of the striatum. The goal of this study is to improve cell replacement therapy and DA innervation in the dorsal striatum by knocking down PlexinC1 via short hairpin RNA (shRNA). For that end, two stable hiPSC lines expressing either a shRNA for PlexinC1, or a control scrambled sequence, were generated by lentiviral transduction followed by FACS sorting. hiPSC were then differentiated into DA neural progenitors for 16 days and the KD efficiency of PlexinC1 validated by qPCR, western blot and immunocytochemistry. To assess the therapeutic potential of these cells, DA neurons derived from control and KD hiPSC lines were transplanted into the midbrain of 6-OHDA lesioned mice. Behavioural and post-mortem histological analysis are currently being performed in these mice. We expect that knocking-down PlexinC1 will improve the proper innervation of the grafted dopamine neurons and reverse motor dysfunction in PD mouse model.

2-D-25 Effects of aging on substantia nigra dopamine neuron excitability and synaptic transmission in mice

Rebecca Howell[1], Michael Beckstead[2]
[1]University of Oklahoma Health Science Center, [2]Oklahoma Medical Research Foundation

The degeneration of substantia nigra dopamine neurons is involved in the etiology for Parkinson's disease, which is characterized by progressive loss of motor and cognitive functions. The biggest risk factors for Parkinson's disease are age and sex; most cases occur after age 60 and prevalence is almost two times greater in men than women. While much research in Parkinson's has focused on the molecular mechanisms underlying dopamine neuron degeneration, very little work has considered the influence of sex and normal aging on the functioning of dopamine neurons, which may contribute to disease risk and presentation. In this work, we studied intrinsic firing properties and synaptic transmission to substantia nigra dopamine neurons with in vivo single unit recordings and whole cell patch clamp recordings in brain slices in male and female C57Bl/6 mice at ages 4, 18 and 24 months. Dopamine neurons recorded from male mice ages 18 months and older showed disruptions in firing activity and an increased propensity to depolarization block compared with younger males, while neurons from females remained largely unchanged across ages. Also, preliminary data suggest enhanced GABAergic transmission in dopamine neurons with age in males; likely a compensatory mechanism to balance the increased dopamine neuron excitability in order to maintain normal motor functioning. Understanding how aging affects dopamine neuron functioning, as well as the balance of excitatory and inhibitory input can help determine vulnerabilities to neurodegenerative processes like those seen in Parkinson's disease

2-D-26 Beta amyloid deposition and cognitive decline in Parkinson's disease: a study of the PPMI cohort

Alexander Mihaescu[1], Mikaeel Valli[1], Carme Uribe[1], Maria Diez-Cirarda[1], Mario Masellis[1], Ariel Graff-Guerrero[1], Antonio Strafella[1]
[1]University of Toronto

The accumulation of beta amyloid in the brain has a complex and poorly understood impact on the progression of Parkinson's disease (PD) pathology and much controversy remains regarding its role, specifically in cognitive decline symptoms. To better understand this relationship, we examined a cohort of 25 idiopathic PD patients and 30 healthy controls from the Parkinson's Progression Marker Initiative database. These participants underwent [18F]Florbetaben positron emission tomography scans to quantify beta amyloid deposition in 20 cortical regions. We analyzed this beta amyloid data alongside the longitudinal Montreal Cognitive Assessment (MoCA) scores across 3 years to see how participant's baseline beta amyloid levels affected their cognitive scores prospectively. The first analysis we performed with these data was a hierarchical cluster analysis where we found that beta amyloid clusters differently in PD patients compared to healthy controls: in the PD group, increased beta amyloid burden in cluster 2 was associated with worse cognitive ability, compared to deposition in clusters 1 or 3. We also performed a stepwise linear regression where we found an adjusted R^2 of 0.495 (49.5%) in a model explaining the PD group's MoCA score one-year post-scan, encompassing the left gyrus rectus, the left anterior cingulate cortex, and the right parietal cortex. Taken together, these results suggest regional beta amyloid deposition, and not global beta amyloid burden, has a moderate effect on predicting future cognitive decline in PD patients. The patchwork effect of beta amyloid deposition on cognitive ability may be part of what separates cognitive impairment from cognitive sparing in PD.

E – Development and diversity of the dopamine systems

2-E-27 Early activation of dopaminergic system alters behavior and neural branching of prepubertal mice in a sexually dimorphic manner

Laila Arabe[1], Muiara Moraes[1], Ana Luiza L. Reis[1], Bruna Resende[1], Sofia Avritzer[1], Paula Valverde[1], Bruno Souza[1]

[1]Universidade Federal de Minad Gerais

It is known that some neuropsychiatric disorders share both dopaminergic (DA) and neurodevelopmental hypothesis. However, little is known about the role of DA signaling in the dynamic of brain development. The first 5 postnatal days (PD) is a developmental window on which there is a high rate of synaptogenesis. Thus, we investigated if DA imbalance during these first 5 PD affects the behavior of mice before puberty. For this, we daily i.p. treated newborn mice with L-Dopa Benserazide, D2 receptor agonist Quinpirole (Qui), D1 receptor agonist SKF-38393 or saline (Sa) from PD1 until PD5. At the age of 30 days, we evaluated exploratory behavior, anxious- and depression-like behavior by open field test (OF), elevated plus maze (EPM), novelty suppressed feeding test (NT) and forced swim test (FST). In addition, we investigated the hippocampal neuronal branching by Golgi-cox method. Females treated with SKF showed a decrease in the number of head dippings on EPM, an increase of the latency time on NT, and a reduction in the hippocampal neuronal branching. Males treated with SKF showed a decrease in the number of stretching on EPM and an increase in the immobility time on FST. Males treated with Qui showed a decrease in distance walked on OF and in climbing time on EPM. In order to investigate if DA signaling is altered on prepubertal mice, we i.p. treated L-dopa Benserazide 30 minutes before behavioral tests. The mice previously treated with L-Dopa during the first 5 PD showed higher behavioral response to the new administration with L-Dopa on OF and FST compared to those previously treated with Sa. Our results suggest a sexual dimorphic function of DA during the early postnatal brain development, affecting prepubertal behavior, brain morphology and dopaminergic signaling.

2-E-28 Regulation of the axonal translatome in dopaminergic circuits during development.

Charles Gora[1], Emmanouil Metzakopian[2], Martin Lévesque[1]
[1]CERVO brain research center, [2]UK Dementia Research Institute at University of Cambridge

Midbrain dopaminergic (mDA) neurons play important roles in controlling a variety of brain functions. An abnormal development of the dopaminergic circuits can lead to various brain disorders. In addition, degeneration of mDA neurons is the leading cause of Parkinson's disease. Dopamine neurons in the midbrain form a heterogeneous set of neurons that innervate different regions of the brain. However, the developmental mechanisms regulating the precise organization of these neuronal circuits remain poorly understood. Recent discoveries have revealed that a proportion of the proteins used for the navigation of developing axons are produced locally in axons. This project aims to reveal the cellular and molecular mechanisms regulating the precise development of dopaminergic sub-circuits. To identify mRNAs locally translated in mDA axons, we used transgenic mice in which ribosomes in mDA neurons are specifically tagged. Using these mice, we isolated mRNAs associated with the ribosomes in mDA axons innervating distinct brain regions and at several developmental time points. Analysis of axonal mRNA content was performed by RNA-sequencing. Our results indicate that specific set of mRNAs are present in axons innervating the striatum, the nucleus accumbens or the prefrontal cortical areas. We are now validating and study the role of axon guidance receptors present in mDA axons innervating the striatum. In addition to uncover the axonal translatome of the dopaminergic system, this project help to better understand the development of the dopaminergic circuits.

2-E-29 Effects of the activation of dopaminergic receptors on motor behavior of zebrafish larvae in different development windows

Beatriz Campos Codo[1], Ana Monteiro de Souza Lima[1], Kevin Farias de Alvarenga[1], Erika Kelmer Sacramento[1], Marco Romano-Silva[1], Bruno Rezende Souz[1]
[1]Federal University of Minas Gerais

Dopamine (DA) synthesis and DAergic receptors expression occur during a prenatal development in mammals. In zebrafish, it is possible to detect DAergic neurons at 18 hours post-fertilization embryos and complete DAergic neuronal groups at 4 days post-fertilization (dpf) larvae. DA is involved in several functions in a developing brain and zebrafish larvae behaviors. We aimed to investigate DAergic the role in zebrafish larvae motor behavior at different windows of early development. We accutely exposed zebrafish larvae to 100uM DA, 10uM SKF (D1R agonist), or 10uM Quinpirole (D2R agonist) at 5, 7, and 14 dpf. The larva were bathed with embryo medium with the drug, and control larvae underwent manipulation procedures with only embryo medium. We recorded and analyzed motor behavior during 5 and 30 min. Data were analyzed using One-Way ANOVA and Tukey posthoc. We found that exposition to DA, SKF, and Quinpirole decreased motor behavior significantly at 5 and 30 min, in all the ages. In the first 5 min of the test, only 5 and 7 dpf larvae exposed to SKF and Quinpirole larvae showed a more significant reduction of motor behavior than DA. When we analyzed motor behavior during 30 min, larvae exposed to DA, SKF, and Quinpirole showed the same reduction of motor behavior. We observe this pattern in 14 dpf larvae at 5 and 30 min. Intracellular signaling in a developing brain is different from an adult brain, and we hypothesized that our results might be explained by how the DAergic receptors act in the circuit dynamics, leading D1R and D2R activation to similar behavioral phenotypes. Therefore we need further studies to understand DAergic pathway mechanisms in a developing brain.

F – Dopamine and affective disorders

2-F-30 Dopaminergic alterations in Alzheimer's disease: A systematic review and meta-analysis

Md Azharuddin[1], Mohammad Adil[1], Pinaki Ghosh[2], Prem Kapur[3], Manju Sharma[1]
[1]School of Pharmaceutical Education and Research, Jamia Hamdard, [2]Poona College of Pharmacy, Bharati Vidyapeeth, [3]Hamdard Institite of Medical Sciences and Research, Jamia Hamdard

Introduction: Low level of Dopamine may contribute to Alzheimer's disease. However, the relationship between Alzheimer's disease and dopaminergic measures remains unclear. Therefore we aimed to perform a meta-analysis to calculate the pooled mean difference (MD) of dopaminergic measures between Alzheimer's disease and control. Methods: A systematic search on, PubMed, and Embase was performed from inception to October 2019. Eligible studies measuring the dopaminergic levels in patients with Alzheimer's disease. Subgroup analysis was performed by the stratification of the dopamine receptors. Heterogeneity was assessed by using Cochrane Q test statistic and inconsistency index (I2). A random effects model was used to calculate the MD with 95% confidence interval (CI) to assess differences in the levels of dopaminergic neurometabolites. Results: Total 10 studies included in this meta-analysis. Results from meta-analysis showed significantly lower levels of dopamine 1 receptor, dopamine 2, and dopamine 3 receptor in patients with Alzheimer's disease compared with controls (MD = -22.78, 95% CI: -35.03, -10.54; $p < 0.003$), (MD = -15.81, 95% CI: -29.03, -2.58; $p < 0.002$), and (MD = -10.50, 95% CI: --20.09, -0.92; $p < 0.03$) respectively. However, there was no significant differences was observed in dopamine 4 and dopamine 5 receptor in patients with Alzheimer's disease compared with controls (MD = -30.32, 95% CI: -109.70, 49.06; p= 0.45), and (MD = 17.62, 95% CI: -15.70, 50.94; p= 0.30). Conclusions: The current finding suggests that low dopaminergic levels were associated with risk of Alzheimer's disease. Moreover, further study needed to robust the present finding.

2-F-31 Role of dopamine D2 receptor in fluoxetine-induced neurogenesis

Gohar Fakhfouri[1], Quentin Rainer[1], Stella Manta[2], Jean-Martin Beaulieu[3], Bruno Giros[1]
[1]McGill University, [2]Paul Sabatier University - Toulouse III, [3]University of Toronto

The selective serotonin reuptake inhibitor fluoxetine is widely prescribed for treating depression. Fluoxetine increases hippocampal neurogenesis and this effect has been associated to some of its chronic antidepressant activity in experimental animals. Since dysregulation of dopamine transmission has been implicated in the pathology of depression and in light of the newly appreciated role of D2R as a risk factor for depression, we examined the role of D2R in the effects of fluoxetine in mice. We demonstrated that chronic antidepressant efficacy and the associated neurogenic effects of fluoxetine require the expression and functionality of brain D2R. Such D2R-dependency of action was specific to fluoxetine and not shared by the monoamine oxidase inhibitor tranylcypromine. Nonetheless, fluoxetine retained its acute behavioral effect in Drd2 knockout mice. This signifies a selective and previously unappreciated role for D2R in pathways associated with neurogenesis and neurogenesis-dependent antidepressive-like behavior. Interestingly, in vitro and in vivo experiments revealed β-arrestin 2 pathway-selective modulation of D2R signaling by fluoxetine. Lastly, our investigation pointed to BDNF as a mediator of D2R-associated effect of chronic fluoxetine in the hippocampus.

2-F-32 Altered intrinsic connectivity within striatal subregions is associated with anhedonia as a function of striatal tissue iron levels among youth with depression

Cecile Ladouceur[1], Teague Henry[2], Amar Ojha[1], Rasim Diler[1]
[1]University of Pittsburgh, [2]University of Virginia

Anhedonia–a core symptom of depression that leads to poor outcomes–is associated with alterations in the dopaminergic reward system, including the striatum. This study examined, among adolescents varying in levels of depression, whether resting state striatal regional homogeneity (ReHo)–an index of intrinsic regional connectivity shown to be reduced in adult depression–was associated with symptoms of anhedonia and to what extent this relationship may be moderated by striatal tissue iron, an index of dopamine(DA) function. Participants (12-17 yrs old, n=75) varying in depression symptoms completed clinical assessments and a resting state fMRI session. ReHo and mean standardized T2* (inverse proxy of tissue iron, itself a proxy for DA concentration) were calculated within the striatum. To examine the relationships between ReHo, mean T2* intensity, and anhedonia symptoms, we used a voxel-wise moderated mediation approach. Results showed that reduced ReHo was associated with higher levels of anhedonia with higher levels of tissue iron concentration in the right caudate (peak T=4.17), and with lower levels of anhedonia in adolescents with lower levels of tissue iron concentration in the same region (peak T=2.99). Lower tissue iron concentration in the left putamen was associated with higher levels of anhedonia overall (peak T=2.79). Findings indicate that intrinsic connectivity in subregions of the striatum is associated with anhedonia but the direction of this relationship is contingent upon striatal dopaminergic function. Such findings point to the need to examine whether dopamine-targeted pharmacotherapy may be effective for a subset of adolescents with anhedonia.

2-F-33 The impact of bupropion on dopamine transporter imaging in a severely depressed patient - how to avoid misdiagnosis of Parkinson´s disease

Lucie Bartova[1], Ivan Milenkovic[1], Konstantinos Papageorgiou[1], Siegfried Kasper[1], Tatjana Traub-Weidinger[1], Dietmar Winkler[1]
[1]Medical University of Vienna

Given the potentially overlapping symptoms as hypomimia, parkinsonoid posture and psychotic features, the diagnosis of Parkinson´s disease in depressed patients may be challenging. We report on a 56-year-old female severely depressed inpatient with anhedonia, indecisiveness, diminished concentration, hopelessness, worthlessness, excessive guilt, delusions and hallucinations. She showed psychomotor retardation, hypomimia and general hypokinesia but postural instability and history of falls. Since antidepressant psychopharmacotherapy including combination/augmentation strategies and electroconvulsive therapy did not improve the clinical picture, the differential diagnosis of Lewy-body dementia was considered. While the cranial magnetic resonance imaging did not show any relevant abnormalities, [123I]FP-CIT-SPECT revealed pathological reduction of nigrostriatal fibers bilaterally and elevated background tracer accumulation in the rest of the brain. As the ongoing antidepressant therapy with the norepinephrine-dopamine reuptake inhibitor bupropion 300 mg/die (bupropion/hydroxybupropion serum blood levels in therapeutic ranges) was noticed, a repetition of [123I]FP-CIT SPECT after a washout phase of 8 days (>4 half-life periods of bupropion lasting 20-37h each) was performed and reported normal (bupropion/hydroxybupropion serum blood levels below therapeutic ranges). Since dopamine transporters (DAT) were shown to be down regulated in depression, which may be potentiated by a psychopharmacotherapy with bupropion, leading to a significant loss of [123I]FP-CIT binding sites and, hence, resulting in a potential misdiagnosis of parkinsonism, the present report should serve as a cautionary note for use of [123I]FP-CIT in depressed patients receiving drugs acting at the DAT.

2-F-34 Individual differences in dopamine reward circuitry impact aggressive profiles

Romain Durand-de Cuttoli[1], Long Li[1], Antonio Aubry[1], C. Joseph Burnett[1], Scott Russo[1]
[1]Icahn School of Medicine at Mount Sinai

Aggression is an adaptive component of social behavior, highly conserved throughout the animal kingdom. Most of the time it serves one main function: to facilitate the procurement and maintenance of key resources in one's environment. Aggression, however, also is a shared symptom of many psychiatric disorders and can become escalated. Thus, understanding the emergence of pathological aggression is a fundamental research question. This study investigates the neural circuits underlying individual differences in aggressive profiles. More specifically, building on our previous findings on the motivational aspects of aggression, we are probing the activity of dopamine reward brain circuits and how it maps onto territorial aggression, aggression-induced reinforcement, and operant-based aggression-seeking. Importantly, taking advantage of a model we recently developed in the lab, we are now able to study cohorts of female mice showing a wide range of territorial aggression, surprisingly close to what we see with male mice. However, female mice that attack in their home cage don't form a place preference for an aggressive-encounter paired chamber nor do they seek aggression in a social operant-based setting. Using a combination of cutting-edge circuit neuroscience approaches (whole brain clearing and imaging, ex vivo and in vivo monitoring of neuronal activity, chemogenetic manipulations), novel complex behavioral paradigms (operant-based aggression seeking) and fine-tuned behavior analysis our goal is to understand the impact of individual differences in aggression and other complex behavior in male and female mice and to identify predictors for the emergence of aggression.

H – Dopamine drug development and pharmacology

2-H-35 Quinpirole-induced suppression of dopamine efflux as a model to assess presynaptic D2 autoreceptor antagonists: preliminary studies with the amisulpride, l-tetrahydropalmatine and l-govadine

Soyon Ahn[1], Haiyan Zou[1], Anthony Phillips[1]
[1]University of British Columbia

Plant-derived tetrahydroprotoberberine (THPB) molecules show promise as novel treatments for neuropsychiatric conditions associated with the dysregulation of dopamine (DA) function. This putative therapeutic efficacy was initially attributed to partial agonism at the D1 and antagonism at the D2 postsynaptic receptors. However, DA activity is also tightly regulated by presynaptic mechanisms at axon terminals that provide inhibitory feedback to terminal DA release. Our recent in vivo microdialysis studies provide compelling evidence that THPBs can modulate basal DA neurotransmission through selective antagonist properties at D2 autoreceptors (D2ARs) in the Nucleus Accumbens (NAc). A reference study confirmed that intra-NAc reverse-dialysis (RD) of the D2 agonist quinpirole (QUI, 0.1-1.0 µM) resulted in a rapid dose-dependent reduction of DA efflux; conversely, the well-characterized D2 antagonist eticlopride (ETI, 50 nM) elicited a significant increase in DA efflux. ETI, administered intra-NAc (50 nM, RD) or systemically (0.3 mg/kg, IP) reversed the QUI-induced suppression of DA efflux. As another positive reference, low doses of amisulpride (2.5, 5.0 mg/kg, IP) shown to have selective effects at D2ARs also reversed QUI-induced suppression of basal DA efflux in the NAc. This QUI assay was then used to screen for D2AR antagonist properties of two THPBs. Both l-tetrahydropalmatine (l-THP; 5 mg/kg, IP or 50 µM, RD) and l-govadine (l-GOV, 1 mg/kg, IP) reversed the effects of QUI and restored DA efflux to near baseline levels. These results are consistent with the hypothesis that both l-THP and l-GOV

can act as potent and selective antagonists at the D2AR, which may have clinical relevance for treating neuropsychiatric conditions arising from a hypodopaminergic state.

2-H-36 Multiplexing dopamine metabolite detection using fast scan cyclic voltammetry

Alexander Zestos[1], Pauline Wonnenberg[1]
[1]American University

Fast scan cyclic voltammetry (FSCV) and carbon-fiber microelectrodes (CFMEs) have been utilized used to detect several important neurochemicals in vivo. However, this method is limited due to the ability to discriminate dopamine from several of its metabolites. Carbon nanotube and polymer modified microelectrodes will be utilized to detect physiologically low levels of neurotransmitters that also resist surface fouling and have high temporal resolution to detect fast changes of neurotransmitters. Furthermore, novel electrode coatings and waveforms will also be utilized to detect several neurotransmitter metabolites such as dopamine, norepinephrine, normetanephrine, 3-methoxytyramine (3-MT), homovanillic acid (HVA), 3,4 dihydroxyphenylacetic acid (DOPAC), and other metabolites. Currently, dopamine is thought to be an important neurotransmitter concerning several disease states such Parkinson's disease, drug abuse, and other. However, dopamine is metabolized on a subsecond timescale, and studies have pointed to the importance of neurotransmitter metabolites in these disease states apart from dopamine. Presently, there is no method to selectively co-detect these neurotransmitter metabolites of dopamine utilizing FSCV. Through several waveform modifications and polymer electrode coatings, we develop a novel method to tune the detection of dopamine and said metabolites, which will help differentiate dopamine and respective metabolites through the shapes and positions of their respective cyclic voltammograms. Measurements have also been made in zebrafish whole brain ex vivo showing the application of this technique in biological tissue. This will have many implications in better understanding complex disease, behavioral, and pharmacological states.

2-H-37 Chirality of novel bivalent dopamine D3 receptor agonists determines bias among G protein subtypes

Hideaki Yano[1], Ana Semeano[1], John Famiglietti[1], Pramisha Adhikari[2], Kuo-Hao Lee[2], Alessandro Bonifazi[2], Francisco Battiti[2], Amy Newman[2], Lei Shi[2]
[1]Northeastern University, [2]National Institutes of Health

The dopamine D3 receptor (D3R) is a well-characterized target for therapeutic development in neuropsychiatric disorders. Its agonists have been studied for movement disorders such as restless leg syndrome and Parkinson's Disease. To improve the outlook of D3R agonist-based medication development, based on a potent D3R-preferential agonist PF592,379, we have designed, synthesized, and pharmacologically characterized bitopic ligands with a secondary pharmacophore (SP) tethered to the PF592,379 scaffold by a linker. We further resolved the chiral center in PF592,379 to yield two diastereomers and made bitopic ligands with them to improve D3R selectivity and affinity. We found that chirality was determined to be a critical component of the most selective D3R agonists in this series. In D3R and D2R functional assays using bioluminescence resonance energy transfer (BRET), most of the ligands showed modest D3R over D2R selectivity and full agonism in D3R GoA protein activation. Some ligands demonstrated an improved G protein coupling profile, while that for β-arrestin recruitment remained the same, thus resulting in significant G-protein activation bias. Intriguingly, stereoisomers of those ligands exhibited considerable differences in potency, efficacy, as well as G protein subtype bias. A pairwise analysis of stereoisomers suggests the linker and SP dictate the differences. Using molecular

modeling and simulation approaches, we identified the structural basis of these differences resulting from the chirality at key positions in the lead compounds. Together, our PF592,379-based bitopic D3R agonists provide novel tools for the preferential activation of G protein subtypes via D3R.

I – Anatomy and physiology of Dopamine systems

2-I-38 Exploring the implication of neurexins in synapse formation and function by dopamine neurons

Charles Ducrot[1], Gregory De Carvalho[2], Benoît Delignat-Lavaud[1], Constantin VL Delmas[3], Nicolas Giguère[1], Sriparna Mukherjee[1], Marie-Josée Bourque[1], Parent Martin[3], Lulu Chen[2], Louis-Éric Trudeau[1]
[1]Université de Montréal, [2]University of California, [3]Université Laval

Midbrain dopamine (DA) neurons are key regulators of basal ganglia functions. The axonal domain of these neurons is highly complex, with a large subset of non-synaptic release sites and a smaller subset of synaptic terminals from which glutamate or GABA are released. The molecular mechanisms regulating the connectivity of DA neurons and their neurochemical identity are unknown. An emerging literature suggests that neuroligins, trans-synaptic cell adhesion molecules, regulate both, DA connectivity and neurotransmission. However, the contribution of their major interaction partners, neurexins (Nrxns) is unexplored. Here we tested the hypothesis that Nrxns regulate DA neuron neurotransmission. Mice with conditional deletion of all Nrxns in DA neurons (DAT::Nrxns KO) were not impaired in basic motor functions. However, they showed an impaired locomotor response to the DA releaser amphetamine. In line with an alteration in DA neurotransmission, decreased levels of the membrane DA transporter (DAT) and increased levels of the vesicular monoamine transporter (VMAT2) were detected in the striatum of DAT::Nrxns KO mice, along with a reduced rate of DA reuptake following activity-dependent DA release. Strikingly, electrophysiological recordings revealed an increase of GABA co-release from DA neuron axons in the striatum of these mice. Together, these findings suggest that Nrxns act as regulators of the functional connectivity DA neurons

2-I-39 Projection-selective effect of Ivabradine on pacemaking in midbrain dopamine neurons with defined axonal targets

Johanna Mankel[1], Joseph Shin[1], Jochen Roeper[1]
[1]Neuroscience Center, Goethe- University

Hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels are prominent in midbrain dopamine (DA) neurons. Previous studies reported significant differences in HCN channel expression patterns and functional properties across defined DA subpopulations (Franz et al., 2000; Neuhoff et al., 2002) including those with distinct axonal projections (Lammel et al., 2008; Lerner et al., 2015). As the subpopulation-specific functional contribution of HCN channels is still unclear among mature DA SN neurons, we combined retrograde tracing of either dorsolateral-striatum (DLS) or dorsomedial-striatum (DMS) with in vitro patch-clamp recordings of synaptically isolated neurons. Using whole-cell voltage clamp, we observed significantly larger current amplitudes for DLS-projecting compared to DMS-projecting DA SN neurons (DLS: 848.9 ± 256.0 pA (n = 32); DMS: 345.6 ± 138.6 pA (n = 25)). To define the role of HCN channels in pacemaking, we recorded DLS- and DMS-projecting DA SN neurons in perforated patch and bath-applied 100 µM ivabradine. To our surprise, ivabradine did not alter pacemaking frequency or regularity in DLS-projecting neurons (pre: 5.31 Hz, CV 5.6%; during: 4.71 Hz, CV 6.72%; post: 4.89 Hz, CV 6.61% (n = 10)) while effectively inhibiting HCN channels. In contrast, pacemaker rate and precision were significantly reduced (approx. 25%) by ivabradine in DMS-projecting DA SN neurons

(pre: 4.02 Hz, CV 9.9%; during: 3.03 Hz, CV 24.15%; post: 3.91 Hz, CV 10.35% (n = 12)). We are currently studying off-target effects (Peters et al., 2021) and fast homeostatic pacemaker dynamics (Amendola et al., 2012) of two DA SN populations to better understand the projection-selective pacemaker effect of ivabradine.

2-I-40 Inhibitory co-transmission from midbrain dopamine neurons relies on presynaptic GABA uptake

Riccardo Melani[1], Nicolas Tritsch[1]
[1]New York University

Dopamine (DA)-releasing neurons in the substantia nigra pars compacta (SNc DA) inhibit target cells in the striatum through postsynaptic activation of γ-aminobutyric acid (GABA) receptors. However, the molecular mechanisms responsible for GABAergic signaling remain unclear, as SNc DA neurons lack enzymes typically required to produce GABA or package it into synaptic vesicles. Here we show that aldehyde dehydrogenase 1a1 (Aldh1a1), an enzyme proposed to function as a GABA synthetic enzyme in SNc DA neurons does not produce GABA for synaptic transmission. Instead, we demonstrate that SNc DA axons obtain GABA exclusively through presynaptic uptake using the membrane GABA transporter Gat1 (encoded by Slc6a1). GABA is then packaged for vesicular release using the vesicular monoamine transporter Vmat2. Our data therefore show that presynaptic transmitter recycling can substitute for de novo GABA synthesis and that Vmat2 contributes to vesicular GABA transport, expanding the range of molecular mechanisms available to neurons to support inhibitory synaptic communication.

J – Dopamine and brain circuitry

2-J-41 Dorsal raphe nucleus dopaminergic neurons project to the orbitofrontal cortex

Duncan Noble[1], Aida Mohammadkhan[1], Min Qiao[1], Stephanie Borgland[1]
[1]University of Calgary

The dorsal raphe nucleus (DRN) is a heterogeneous region in the pons consisting of dopamine and serotonin neuromodulators, as well and glutamate and GABAergic neurons. The DRN serotonergic projection is the largest contributor of serotonin thought the cortex and has been well studied, however the dopaminergic afferent projections or effects are not well known. The orbital frontal cortex (OFC) is known for evaluation of rewarding stimuli. Mesolimbic dopaminergic neurons relay information on the relative predictive value of rewarding stimuli to the OFC. However, it is unknown whether dopamine neurons of the DRN project to the OFC. We therefore examined the DRN dopaminergic projection to the OFC. DATcre Tdtomato mice were injected with 488 CTB into the left lateral or medial OFC and the CTB and Tdtomato colocalizated neurons were quantified. In addition, an anterograde AAV2/8 Ef1a DIO eYFP was administered into the DRN and eYPF fibers were imaged in the medial and lateral OFC. The number of DRN dopaminergic neurons projecting to the medial or lateral OFC injections, identified by colocalization of CTB with Tdtomato, were not significantly different. Similarly, the percentage of the total dopaminergic population did not significantly differ between the medial or lateral OFC. On average 8% of DRN dopamine neurons project to the OFC. AAV-DIO-eYFP injection showed 86% efficiency of expression within DRN dopamine neurons and eYFP labeled fibres were present in lateral and medial fibers in the OFC. Overall, dopamine from the DRN project to the medial and lateral OFC. Future work will identify the functional properties of DRN dopamine in the OFC.

2-J-42 Disambiguating local connectivity of non-dopaminergic projection neurons in the Ventral Tegmental Area

Lucie Oriol[1], Thomas Hnasko[1], Sarah Uran[1]
[1]UCSD

The Ventral Tegmental Area (VTA) has a crucial impact on reward-related behaviors, furthermore, the mechanisms of action of addictive drugs converge at the VTA with a common effect of increasing the dopamine released from VTA projections, most famously in the nucleus accumbens (NAc). Understanding the VTA's functional organization is therefore required to better characterize the mechanisms underlying addiction. For example, opioids reduce inhibition in the VTA, thereby disinhibiting dopamine neurons (Johnson and North 1992) which contributes to the reinforcing properties of those drugs. While many assume dopamine disinhibition in VTA is mediated by GABA interneurons (classically defined as neurons only making local connections such that their soma and axon are contained in the same brain region), there is no direct evidence that interneurons exist in VTA. Instead, GABA projection neurons in VTA could make intra-VTA collaterals. To determine whether VTA GABA and glutamate projection neurons make local connections, we used optogenetics-assisted electrophysiology to functionally identify local GABA and glutamate connections made by VTA neurons defined by their projection target. We selectively expressed ChR2 in VTA projection neurons (to NAc, Prefrontal Cortex, and Ventral Pallidum successively) and performed whole-cell recordings of VTA neurons. We detected opto-evoked excitatory postsynaptic currents, presumably mediated by glutamate, and opto-evoked inhibitory postsynaptic currents, presumably mediated by GABA. Our results suggest that VTA projections neuron collateralize to make intra-VTA connections (as well as distal ones) which could mediate VTA dopamine neuron disinhibition and support functions attributed to putative VTA interneurons.

2-J-43 Spatiotemporal relationships between dopamine and acetylcholine dynamics across the striatum during classical conditioning

Safa Bouabid[1], Mai-Anh Vu[1], Mark Howe[1]
[1]Boston University

In the striatum, both acetylcholine (Ach) and dopamine (DA) respond to salient stimuli, unpredicted rewards and reward predicting cues. Recordings in behaving animals indicate that these neuromodulators have coincident phasic changes but in the opposite directions, suggesting that they may play opposing roles in controlling aspects of saliency and learning. However, mechanistic studies in-vitro have found a more complex relationship in which striatal DA and Ach release reciprocally co-modulate each other both antagonistically and synergistically via different receptor subclasses. Although the complex interplay of DA and Ach in the striatum has been heavily studied in brain slices, much less is known about how naturally occurring DA and Ach signals are simultaneously regulated in-vivo across the striatum to mediate behavior and learning. We used genetically encoded fluorescent sensors and a dual-color imaging strategy in combination with a novel optical approach to chronically measure DA and Ach levels across over 100 striatum locations simultaneously during classical conditioning. Both neuromodulators displayed significant, and sometimes unexpected, spatial heterogeneities in signaling to cues and rewards across striatum locations. Relationships between DA and Ach signaling were not fixed, but instead reflected correlations in timing and amplitude consistent with both synergistic and antagonistic interactions, depending on striatum region and behavioral event. Significant variations were also observed in slower timescale changes in DA and Ach signals across the striatum over days

during learning. Overall, our findings advance a comprehensive understanding of how striatum-wide DA and Ach dynamics are coordinated during behavior on multiple temporal and spatial scales.

2-J-44 Cell-type specific influence of reduced striatal dopamine signalling in vivo

Thomas Christinck[1], Christopher Lafferty[1], Angela Yang[1], Thalia Garvock-de Montbrun[1], Milan Valyear[1], Jonathan Britt[1]
[1]McGill University

Dopamine signalling in the striatum regulates the excitability of striatal spiny projection neurons (SPNs). In cell culture preparations, dopamine antagonistically regulates striatal subpopulations through D1 and D2 receptor signaling which increases and decreases SPN excitability, respectively. However, in vivo experiments have suggested that the influence of dopamine signalling in the intact network is more complicated. More specifically, the extent to which dopamine differentially regulates the relative and absolute activity levels of striatal SPN subpopulations in vivo is unclear. Here we investigate how transient, optogenetic manipulations of midbrain dopamine neuron activity affect D1 and D2 SPN excitability. Soma-restricted opsin expression was targeted to midbrain dopamine neurons in transgenic mice, while calcium-sensitive fluorescent indicators of neural activity were expressed in D1 or D2 SPNs in lateral nucleus accumbens or dorsal striatum. Cell type-specific responses to dopamine neuron manipulations were recorded during locomotion and rest in an animal's home cage or in a novel environment. We find that dopamine's influence on D1 and D2 SPNs is consistent across striatal subregions and dependent on contextual variables, including state of arousal and motivation. Our data suggest that dopamine signalling influences the absolute activity of these populations in a cooperative manner, indicating that dopaminergic modulation of SPN activity in vivo is not as antagonistic as current models based on in vitro work would predict.

K – Dopamine receptors, transporters & signalling

2-K-45 Exocyst-dependent trafficking of dopamine transporter and its mutants linked with infantile parkinsonism dystonia

Hafiz Muhammad Mazhar Asjad[1], Michael Freissmuth[1], Sonja Sucic[1]
[1]Medical University of Vienna

The transfer of material between organelles is mediated by carrier vesicles. Each vesicle transport reaction can be divided into four essential steps: vesicle budding, transport, tethering, and fusion. The exocyst is a multiprotein complex required by many membrane proteins for delivery to and insertion into the plasma membrane. Uptake through the neurotransmitter transporters for example dopamine transporter (DAT) represents the primary mechanism used to terminate dopaminergic transmission in the brain. However, little is known about the specialized trafficking of DAT towards the target membrane. DAT requires an intact C-terminal PDZ-binding motif to reach the cell surface, whereas the closely related serotonin transporter SERT does not. Here, we tested the hypothesis that DAT requires the exocyst for reaching the cell surface. HEK 293 or CAD cells were transiently co-transfected with plasmids encoding the wild-type dopamine transporter (DAT) and serotonin transporter (SERT) along with different components of the exocyst, i. e. Exo70, Sec6 and Sec8 using jetPRIME (Polyplus). Radioligand uptake, confocal laser scanning microscopy and immunoprecipitation experiments were performed 48 h after transfection to study the effect of exocyst components on trafficking of DAT and SERT. DAT relied on the exocyst to reach the cell surface. Surprisingly, SERT did not require the exocyst complex to reach the cell surface, regardless of whether the experiments were performed in HEK 293

cells or in CAD cells (a Cath.a-cell-derived line of neuronal origin) membrane. We found that two components of the exocyst complex, Sec8 and Exo70, separately control the trafficking of DAT. Immunoblots also showed the effect of exocyst components on trafficking of DAT as control.

2-K-46 Projection-specific hierarchical organisation among midbrain dopamine neurons via dopamine autoreceptor inhibition

Niklas Hammer[1], Sanghun Lee[1], Beatrice Fischer[1], Strahinja Stojanovic[1], Jochen Roeper[1]
[1]Goethe University Frankfurt

Most midbrain dopamine (DA) neurons are inhibited by activation of somato-dendritic D2-autoreceptors (D2R) (Lacey et al., 1987). The presence of synaptic D2R signalling between pre- and postsynaptic DA neurons was demonstrated in midbrain slices in vitro (Beckstead et al., 2004). In addition, spontaneous D2R-mediated IPSCs were recorded in DA neurons showing functional dendro-dendritic vesicular DA release between midbrain DA neurons (Gantz et al., 2013). Our previous studies reported different D2R and GIRK2 expression levels in midbrain DA neurons projecting to distinct target areas (Lammel et al., 2008), but the functional contribution of synaptic and extrasynaptic D2R signaling within and across these DA subpopulations is currently unknown. Therefore, we recorded electrically evoked, D2R-mediated, sulpiride-sensitive, slow inhibitory postsynaptic currents (eIPSCs) in retrogradely identified DA neurons in vitro. We observed significant differences in peak D2R-sIPSC amplitudes between DA neurons projecting lateral shell of nucleus accumbens (lNAcc), dorsomedial striatum (DMS) and dorsolateral striatum (DLS). Mean eIPSC amplitudes of lNAcc-projecting DA neurons displayed much larger currents compared to DA neurons projecting to the dorsal striatum (lNAcc:27.1±2.5pA, n=21; DMS:16.3±1.4pA, n = 23; DLS:12.5±1.3pA, n=18). Using a viral approach we optogenetically stimulated presynaptic DA neurons projecting to DLS while recording optically-evoked, sulpiride-sensitive D2-IPSC (oIPSC) in DA neurons projecting to lNAcc (lNAccDLS:14.9±3.7pA, n=13). When reversing pre- and postsynaptic DA neurons, little to no oIPSCs were recorded (DLSlNAcc:3.6±1.4pA, n=9). These results suggest a lateral-to-medial hierarchical organisation of D2R-inhibition among distinct DA subpopulations.

2-K-47 Tetrahydrocannabinol (THC) in adolescence dysregulates the signaling pathway that orchestrates dopamine development

Tanya Capolicchio[1], Giovanni Hernandez[1], Estrada Katerina[1], Emilie Dube[1], Cecilia Flores[1]
[1]McGill University

Adolescence is a period of dynamic mesocorticolimbic dopamine developing, when axons of dopamine neurons that originate in ventral tegmental area (VTA) grow from the striatum towards the prefrontal cortex (PFC), ultimately fine-tuning adult PFC function and cognitive control. This protracted growth is controlled by the coordinated activity of Netrin-1 and its receptor gene,Dcc. Previous research from our lab showed that amphetamine in adolescence in male mice alters Dcc in the VTA and leads to deficits in cognitive control in adulthood. Here we assessed whether adolescent exposure to the main psychoactive component of cannabis, THC, alters Dcc mRNA and its microRNA repressor, miR-218, in dopamine neurons, and modifies aspects of cognitive control in adulthood. Male and female C57/Bl6 mice (PND 22) received intraperitoneal injections of THC (0, 2.5, 5, mg/kg) once every other day, for 10 days. One week later, VTA miR-218 and Dcc levels were quantified. A separate cohort was tested in adulthood in the Go/No-Go task. Males exhibit decreased miR-218 expression and a concomitant increase in Dcc levels. These changes were associated with improved stop impulsivity, but impaired wait impulsivity. In females, THC in adolescence decreases Dcc without altering miR-218, does not impact

adult stop impulsivity, but impairs waiting impulsivity. THC in adolescence may therefore alter mesocorticolimbic dopamine development, in a sex-specific manner, by inducing opposite changes in the Netrin-1/DCC pathway and by recruiting different epigenetic processes.

2-K-48 Dopamine Drives neuroHIV Neuropathogenesis by Increasing Myeloid Infection and Inflammation

Peter Gaskill[1], Emily Nickoloff-Bybel[2], Stephanie Matt[1], Rachel Nolan[3], Breana Channer[1], Alexis Brantly[1]
[1]Drexel University College of Medicine, [2]University of Pennsylvania, School of Dental Medicine, [3]University of Washington School of Medicine

Myeloid cells are one of the primary cell types targeted by HIV and one of the main drivers of HIV neuropathogenesis. HIV infection, especially in the CNS, disrupts dopamine regulation, exposing myeloid cells to aberrantly high dopamine concentrations. This can be amplified by substance misuse or use of dopamine altering therapeutics. Our data indicates that activation of dopamine receptors on myeloid cells can potentiate HIV pathogenesis by both increasing HIV infection of macrophages and promoting inflammation mediated by the macrophage population. Activation of human myeloid dopamine receptors does not alter cAMP production, but activates a non-canonical signaling pathway that increases Ca2+ flux and PKC phosphorylation. These changes are, at least partially, mediated through a G□q-activated pathway, suggesting that D1-like receptors, and potentially D5, are the primary receptors initiating this signaling. Activation of this pathway results in increased HIV replication in myeloid populations through increases in viral entry that are induced by increases in Ca2+ release. Activation of PKC, along with activation of Akt, also seems to increase myeloid production of inflammatory factors, by increasing the nuclear translocation of NF-□B. This results in an increase in inflammatory mediators such as IL-6 and CXCL-10, as well as priming the NLRP3 inflammasome, predisposing individuals with disrupted dopamine regulation to aberrant inflammatory responses. These data show that dopamine enhances viral replication and inflammation, potentially increasing the size of the viral reservoir and promoting further tissue damage in the brain, and this could be exacerbated in individuals misusing addictive or therapeutics that modulate the dopaminergic system.

2-K-49 Dopamine-driven increase in IL-1b in myeloid cells is mediated by differential dopamine receptor expression and exacerbated by HIV

Stephanie Matt[1], Breana Channer[1], Emily Nickoloff-Bybel[1], Samyuktha Manikandan[1], Yash Agarwal[1], Joanna Canagarajah[1], Kaitlyn Runner[1], Rachel Nolan[1], Peter Gaskill[1]
[1]Drexel University College of Medicine

Neurological complications of HIV infection, known as neuroHIV, remain prevalent even in individuals on antiretroviral therapy (ART). Data suggest that neuroinflammation is central to HIV neuropathogenesis and can be exacerbated by substance use disorders (SUDs), which are highly comorbid with HIV. Despite distinct mechanisms of action, all substances of abuse increase dopamine, suggesting that dopamine is a common mechanism by which addictive drugs potentiate neuroHIV. Our published data show that dopamine increases inflammatory cytokine production such as IL-1β in human microglia and macrophages, but the mechanisms are not clear. Studies indicate that D1-like dopamine receptors mediate inflammatory activity while D2-like dopamine receptors mediate anti-inflammatory activity, and we have shown that human macrophages and microglia have higher D1-like receptor expression. Therefore, we hypothesize that dopamine-mediated IL-1β in myeloid cells is regulated by different dopamine receptor ratios and can be exacerbated by HIV. Our data in macrophages indicate that DRD1

is necessary for but that changes in DRD2 can alter the magnitude of dopamine-mediated increases in IL-1β. We confirm this in human microglia cell lines, showing that dopamine only increases IL-1β in microglia with a high D1-like/D2-like ratio and that antagonizing dopamine receptor expression diminishes this effect. We also show that the effects of dopamine on IL-1β are potentiated in the presence of HIV infection in both microglial cell lines and iPSC-derived microglia. Due to the prevalence of both SUDs and use of dopamine-modulating therapeutics, a detailed understanding of dopamine-mediated changes in inflammation will be critical to effectively tailor ART to HIV-infected individuals using these drugs.

2-K-50 Next generation RNA sequencing transcriptomic analysis in wild-type and Nur77 (Nr4a1) deficient rats reveals novel signalling components modulated by haloperidol

Simon Majeur[1], Giovanni Hernandez[2], Catherine Lévesque[1], Claude Rouillard[3], Pierre Blanchet[1], Daniel Lévesque[1]

[1]University of Montreal, [2]McGill University, [3]Laval University

Despite antipsychotic drugs are used for several decades, their mechanism of action beyond their interaction with dopamine and serotonin receptors remains elusive. Nur77 (Nr4a1) is a transcription factor of the nuclear receptor family associated with antipsychotic drug effects. However, the mechanism of action of Nur77 is also not well understood. To better understand the signalling components implicated with antipsychotic drug use and Nur77 activity, we compared striatal gene transcripts following haloperidol in wild-type and Nur77-deficient rats using Next Generation RNA Sequencing (RNAseq) and bioinformatic analysis. Haloperidol and Nur77 deficiency modulated important subsets of striatal genes associated with dopamine receptor signalling and glutamate synapses. The analysis revealed modulations of key components of G protein signalling that are consistent with a rapid adaptation of striatal cells that may explain, at least partially, haloperidol-induced dopamine D2 receptor upregulation and supported the increase of the percentage of high vs low affinity states of the D2 receptor observed in Nur77 knockout mice. Amongst significantly modulated transcripts, dual specificity phosphatase 5 (Dusp5) represents a new and very interesting candidate. Indeed, we confirmed that putamen Dusp5 protein levels were associated with abnormal involuntary movements in non-human primates chronically exposed to haloperidol. This transcriptomic analysis showed important and rapid haloperidol-induced G protein-coupled receptor signalling alterations that may support prolonged cellular adaptations and identified, for the first time, a putative Nur77-dependent expression of Dusp5 as a new signalling component associated with antipsychotic drug-induced tardive dyskinesia.

M – Dopamine and behavior

2-M-51 The role of the tail of the striatum in safety learning

Adrien Stanley[1]

[1]Columbia University

Distinguishing between safety and danger is critical for survival. Impairments in perceived safety can lead to disorders of excess fear, such as post-traumatic stress and anxiety disorders(PTSD). PTSD affects 3.6% and anxiety disorders affect 19.1% of the United States population. Treatments for these conditions are not always effective and have unwanted side effects. The neural circuitries underlying perceived safety from danger remains elusive, and understanding this can help develop targeted therapeutic strategies for treating these disorders. Recent research has begun to implicate striatal

activity, specifically in the tail of the striatum (TS) in safety processing. However, the exact role the TS plays, as well as the role of the various cell subtypes and neuromodulators, in safety responding remains unknown. Neuromodulators that could alter TS activity are serotonin and dopamine. Impairments in serotonergic signaling has long been implicated in the pathogenesis of anxiety, and serotonin signaling has been shown to influence learned safety and avoidance behavior. This suggests that serotonergic modulation of striatal activity may influence safety responses. Serotonin may alter safety responses via an interaction with dopamine, as serotonergic signaling in the striatum has been shown to influence dopamine release. The proposed project aims to investigate this by first characterizing activity of striatal cell types during paradigms that involve learned safety responses in mice. Next, I will determine how modulating the activity of these cell types pharmacologically will influence safety responses.

2-M-52 Implication of medial prefrontal cortex and nucleus accumbens dopamine transmission in goal-directed behaviors: a role for dopamine and NMDA receptors heteromers ?

Anna Petitbon[1], Andrea Contini[1], Roman Walle[1], Rodrigue Ortole[1], Javier Correa Vazquez[1], Romain Thebeaud[1], Mélanie Depret[1], Andry Andrianarivelo[2], Jacques Barik[3], Peter Vanhoutte[2], Pierre Trifilieff[1]
[1]Université de Bordeaux, [2]Sorbonne Université, [3]Université Côte d'Azur

Mesocorticolimbic dopamine transmission is believed to be a key modulator of goal-directed actions and reward processing through its action on dopaminoceptive neurons expressing either D1 (D1R) or D2 receptors (D2R) in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc). Through chemogenetic approaches coupled with operant conditioning tasks, we demonstrated an implication of dopaminergic projections from the Ventral Tegmental Area together with a complex interplay between D1R- and D2R-expressing neurons of the mPFC and the NAc in the flexible expression of food-oriented action as well as in the motivation to obtain a palatable reward. In both structures, the activity of dopaminoceptive neurons is strongly regulated by the convergence of glutamatergic and dopaminergic inputs. Heteromers formed by dopaminergic and glutamatergic N-methyl-d-aspartate receptors (NMDAR) recently emerged as molecular coincidence detectors of these transmissions, notably in the context of psychostimulant-induced adaptations. We therefore asked whether these receptor complexes could play a role in physiological processing. Expression of heteromers was mapped in substructures of the striatum and mPFC with Proximity Ligation Assay. Blockade of D1R-NMDAR or D2R-NMDAR heteromerization in either the mPFC or the NAc through the local viral-mediated expression of interfering peptides induced alterations of discrete components of goal directed behaviors. These findings contribute to decipher the role of dopaminoceptive neurons in executive functions and support a key role of heteromers formed by dopamine and NMDA receptors in such processes.

2-M-53 Identification of action prediction error, a value-free dopaminergic teaching signal that drives stable associations in the tail of the striatum

Francesca Greenstreet[1], Hernando Martinez Vergara[1], Sthitapranjya Pati[1], Laura Schwarz[1], Matthew Wisdom[1], Yvonne Johansson[1], Marcus Stephenson-Jones[1]
[1]University College London

Experience dependent changes in corticostriatal plasticity are dopamine-dependent and critical for reinforcement learning. Supporting this, selective frequency-specific corticostriatal plasticity develops in the tail of the striatum (TS) as mice learn auditory discriminations. However dopamine signals in the TS are not related to reward or predicted value, raising the question of how reward-guided associations are formed in this region. Here we first use photometry to show that dopamine in the TS encodes an action

prediction error, a novel value free teaching signal that could be used to reinforce stable sound-action associations. Consistent with a prediction error the responses are stimulus specific and decrease over training as the action becomes predicted by the auditory stimulus. Inspired by computational models of habit formation, we show our data is consistent with a model where dopaminergic input to the TS forms the value-free half of a dual value-based/value-free dopaminergic learning system. In line with this model, chronic lesions of the TS, or the TS-projecting dopaminergic cells, specifically impair later stages of learning. Supporting this, optogenetic inhibition of either the direct or the indirect pathways in TS specifically disrupts behavioural performance in later stages of learning. These results are predicted by our dual controller model where initial learning is driven by a value-based system and then rapidly consolidated in a value-free manner in the TS. Together we show a novel action-based teaching signal works in concert with canonical value-based teaching signals to form a dual dopaminergic teaching system that can consolidate stable state-action associations in the sensory tail of the striatum in a value-free manner.

2-M-54 Establishing a spatial map of dopamine signals during the learning and updating of distinct instrumental associations

Eleanor Brown[1], Mai-Anh Vu[1], Chinyere Godfrey-Nwachukwu[1], Mark Howe[1]
[1]Boston University

Dopamine (DA) signaling in the striatum, the main input to the basal ganglia, is critical for instrumental learning, a process involving associations of stimuli, actions, and outcomes. While DA signals are widely believed to encode reward prediction errors which drive associative learning, evidence suggests that DA release across striatum regions differently encodes variables such as stimuli, action choice, and reward history. Further, behavioral studies suggest that anatomically segregated subregions of the striatum support distinct associative learning and decision-making processes. Thus, subregion-specific DA signals may enable the learning of distinct stimulus, response, and outcome associations. To investigate how spatially varying DA dynamics may contribute to distinct associative learning processes, we have developed a behavioral paradigm which requires mice to switch between a choice strategy based on recent action-outcome associations and a strategy based on fixed stimulus-action associations. We are applying a new, large-scale, multi-optical fiber photometry method to measure DA release dynamics throughout the volume of the striatum as mice learn and update distinct stimulus, action and outcome contingencies. Using linear modeling, we have begun to establish the influence of task and decision variables on DA signals to cues, actions, and rewards across more than 50 sites throughout the striatum. Preliminary findings indicate spatial variations in DA signaling on multiple spatial scales across all three striatal axes related to current and previous action choices and outcomes.

2-M-56 Role of Dopamine Neurons in Familiarity

Rhonda Kolaric[1], Jacquelyn Tomaio[1], Sixtine Fleury[1], Andreas Toft Sørensen[2], Ulrik Gether[2], Susana Mingote[1]
[1]City University of New York, [2]University of Copenhagen

It is well established that ventral midbrain dopamine (DA) neurons increase bursting activity to signal novelty and decrease after repeated stimulus presentations, providing a physiological signature that a stimulus has become familiar. Here, we hypothesized that decreasing DA neuron activity is sufficient to drive rapid familiarization. To test this hypothesis, we used a Novel Object Recognition (NOR) paradigm where mice were presented with two identical objects during a familiarization session, then

subsequently presented with a new object and one previously seen object. Time exploring the new object provides an index of recognition for the familiar object. Further, this exploration time is dependent on the amount of pre-exposure to the familiar objects. One familiarization session is not sufficient to increase exploration of a novel object; equal attention allocated to both stimuli is likely driven by the presence of increased DA neuron burst activity. To investigate whether decreasing DA neuron activity during a single familiarization session prompts gain-of-function in NOR, we chemogenetically inhibited DA neurons in the ventral tegmental area (VTA) during familiarization. TH-flp mice receieved an AAV8 flp-dependent hM4Di into the VTA. We then stimulated hM4Di receptors via CNO and found that inhibiting DA neurons during familiarization increased subsequent novel object exploration. Inhibiting DA neurons after the familiarization session produced no improvement in novel object discrimination. These results suggest that suppressing DA neuron activity ameliorates novelty discrimination by enhancing familiarity recognition. Ongoing experiments suggest that this DA-dependent effect on familiarity may be due to a facilitation of object-context association.

N- Other

2-N-57 Inorganic clay nanocomposite system for improved cholinesterase inhibition and brain pharmacokinetics of donepezil

Anurag Singh[1], Santosh Singh[1]
[1]*Institute of Medical Sciences, Banaras Hindu University*

Objective: To analyze the pharmacokinetics/pharmacodynamics parameters of laponite (LAP) nanocomposites: an effective approach for neurodegenerative disorder management. Significance: Based on the outcomes of this study and taking into consideration of the unique characteristics of laponite, it can be further explored to deliver many other central nervous system acting drugs. Methods: In the present study, laponite (LAP) nanocomposites were exploited for the improved brain delivery of donepezil (DZ) following encapsulation approach due to their nano-size and positive charge at pH < 9. Result: The size of prepared nanocomposites was 53.7 ± 4.0 to 137.7 ± 11.0 nm. The drug was released in a sustained manner till 120 h in phosphate buffer saline (pH 7.4) and acid phthalate buffer (pH 4.0). LAPDZ formulations inhibited acetylcholinesterase approximately by 82%, significantly higher ($p < 0.05$) than plain DZ (30%). Swiss albino mice exhibited enhanced brain uptake of LAPDZ administered via intravenous route. Promising pharmacokinetic parameters were observed in animals treated with LAPDZ. LAPDZ formulation showed half-life (t½), volume of distribution (Vd) and clearance (Cl) as 5.53 ± 0.40 h^{-1}, 0.129 ± 0.02 L, 0.015 ± 0.002 L/h, respectively. While DZ solution showed the same parameters as 1.06 ± 0.12 h^{-1}, 0.168 ± 0.01 L, 0.106 ± 0.013 L/h, respectively. The brain uptake of LAPDZ formulation was improved with quintuplet t½. Conclusion: Based on the results of present study, it is proposed that the formulated nanocomposite would result in improved patient compliance with therapeutic effect at lower doses.

Poster Session 3

A – Dopamine, motivation, reward and addiction

3-A-1 Amphetamine-mediated decoupling of midbrain neurons firing from striatal dopamine release

Mahalakshmi Somayaji[1], Eugene Mosharov[1], David Sulzer[1]
[1]*Columbia University*

Amphetamine (AMPH) and its derivatives are highly addictive substances that elicit their response by increasing striatal extracellular dopamine (DA) levels. Mechanisms that have been suggested to account for this include depletion of DA vesicular stores and blockade and reversal of dopamine uptake transporters (DAT) thus promoting non-exocytotic DA efflux from striatal DA terminals. Additionally, in vivo studies suggest that AMPH augments action potential- mediated presynaptic DA release (Ramsson et al., 2011). Here, we studied the relationship between the firing activity of SNpc dopaminergic neurons and DA release in the dorsal striatum evoked by midbrain electrical stimulation in vivo in anesthetized animals. We found a ~5-fod increase in evoked striatal DA release accompanied by a ~50% decrease in DA neurons spontaneous firing following 10 mg/kg i.p. AMPH injection. The effect of AMPH on both cell bodies activity and striatal DA release appears to be calcium- independent. Furthermore, deficiency of alpha-synuclein - a protein implicated in Parkinson's Disease - diminishes amphetamine-mediated increase in striatal evoked DA release without significantly altering midbrain neurons firing activity. Although still preliminary, these results evaluate the relationship between the activity of DA neurons and DA release from their striatal terminals in vivo, and will help to better understand the mechanisms of AMPH and alpha-synuclein involvement in these processes.

3-A-2 Examining the impact of experience-induced ventral tegmental area KCC2 downregulation on dopamine signaling and reward-related behaviors

Joyce Woo[1], Hannah Kugler[1], Caroline Swain[1], Alexey Ostroumov[1]
[1]Georgetown University

It is widely assumed that ventral tegmental area (VTA) GABA neurons suppress dopamine signaling and reward-related behaviors. However, some evidence suggests that increased GABAergic input onto DA cells correlates with potentiated behavioral responses to rewards. Here, we provide a mechanistic insight into how VTA GABA neurons can shape dopamine signaling and acquisition of reward-related behaviors. First, we describe a novel form of experience-dependent inhibitory synaptic plasticity that enhances excitability of VTA GABA circuitry. Specifically, stress and addictive drugs alter synaptic inhibition of VTA GABA neurons via downregulation of KCC2, a chloride transporter that maintains low intracellular chloride concentrations in neurons. Because low intracellular chloride underlies GABAA receptor-mediated inhibition, KCC2 dysfunction reduces synaptic inhibition and can even cause paradoxical GABAergic excitation of VTA GABA neurons. At the circuit level, we show that experience-induced KCC2 down-regulation in GABA neurons have different impact on separate VTA circuits. Finally, we examine the role of VTA KCC2 and GABA neurons in dopamine-dependent motivated behaviors. In summary, we characterize a novel form of experience-induced synaptic plasticity, which enhances activity of VTA GABA neurons and alters reward-related behaviors.

3-A-3 Effect of modulating dopaminergic and glutamatergic transmission in the nucleus accumbens shell on Pavlovian responding to alcohol cues

Milan Valyear[1], Ghislaine Deyab[2], Soraya Lahlou[2], Alexa Brown[1], Nina Caporicci-Dinucci[2], Iulia Glovaci[3], Andrew Chapman[1], Nadia Chaudhri[1]
[1]Concordia University, [2]McGill University, [3]University of Oslo

Responding triggered by a discrete alcohol-predictive conditioned stimulus (CS) is elevated in a context associated with alcohol relative to a neutral context, and this elevation requires activity in the dopaminergic projection from the ventral tegmental area (VTA) to the nucleus accumbens shell (NAcS). Here, we determined if elevating dopaminergic or glutamatergic transmission in the NAcS would

increase responding to an alcohol CS. In separate groups of male, transgenic TH::Cre rats expressing hM3Dq designer receptors in the VTA, we tested responding to an alcohol CS after systemically administering clozapine-n-oxide (CNO; 10 mg/kg i.p.) or microinfusing CNO (3 mM, .3 µl/hemisphere) directly into the NAcS. In male, wild-type rats we microinfused α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; 0 or 1 mM, .3µl/hemisphere) in the NAcS before tests. Neither systemic administration nor CNO microinfusion in the NAcS affected CS responding. However, food consumption was reduced by systemic CNO in a follow-up experiment, showing that the chemogenetic manipulation was effective, and we also found that CNO-activation of hM3Dq on dopaminergic processes in the NAc increased the amplitude of postsynaptic potentials in medium spiny neurons in vitro. AMPA microinfusion selectively reduced CS responding in an alcohol context but had no effect on CS responding in a neutral context. In summary, increasing dopaminergic activity in the NAcS was insufficient to elevate responding to an alcohol CS, while elevating glutamatergic transmission in the NAcS selectively reduced CS-responding in the alcohol context. These results confirm a role for the NAcS in modulating the impact of context on responding to an alcohol CS.

3-A-5 The interpeduncular nucleus acts as a brake to limit the effect of nicotine on dopaminergic neurons in the ventral tegmental area

Joachim Jehl[1], Eléonore Vicq[1], Maria Ciscato[1], Nicolas Guyon[1], Philippe Faure[1], Alexandre Mourot[1]
[1]ESPCI Paris

Nicotine drives reinforcement primarily by activating nicotine acetylcholine receptors (nAChRs) on dopaminergic (DA) neurons in the ventral tegmental area (VTA). But nicotine also acts in concert on the interpeduncular nucleus (IPN) to induce aversion to the drug. Yet, it is not known how the IPN responds to nicotine in vivo, and whether this response influences nicotine's reinforcing effect in the VTA. Here we used in vivo electrophysiological and fiber photometry recordings in adult mice, coupled with a novel chemogenetic strategy for manipulating specific nAChR subtypes, to probe nicotinic transmission in the IPN and its influence on VTA DA neurons. We show that nicotine activates and inhibits two different populations of IPN neurons starting at low doses that elicit no response in VTA DA neurons. To pharmacologically dissect these effects of nicotine, we developed two novel mutant mouse models, that allow chemogenetic inhibition of specific nAChR subtypes (namely ß2- and ß4-containing receptors). These mice contain a single aminoacid substitution on the beta subunit (E61C), which does not alter receptor function, but allows the covalent attachment of an irreversible antagonist (called MPEG4Ch). Infusion of MPEG4Ch in the IPN of the mutant mice, but not of WT mice, leads to potent, pharmacologically-specific and sustained receptor antagonism. Using these mice, we show that β2 and ß4 nAChRs differentially contribute to nicotine-induced activation and inhibition. Furthermore, we show that blocking IPN β4 nAChRs significantly reduces the threshold response to nicotine in VTA DA neurons. Together, our results suggest that the IPN can facilitate nicotine action on VTA DA neurons, which is likely to alter nicotine reinforcement.

3-A-6 Chemogenetic sensitization of midbrain dopamine neurons exacerbates cue-provoked risk taking and amplifies cocaine self-administration

Tristan Hynes[1], Chloe Chernoff[2], Kelly Hrelja[2], Maric Tse[2], Graeme Betts[2], Melanie Lysenko-Martin[2], Brittney Russell[2], Lucas Calderhead[2], Andrew Li[2], Stan Floresco[2], Catharine Winstanley[2]
[1]iCORD, [2]University of British Columbia

Repeated exposure to psychostimulants, uncertain rewards and reward-predictive cues can all sensitize the dopamine system. The comorbidity between psychostimulant abuse and gambling disorder may therefore theoretically arise because both drug taking and gambling characteristics act synergistically to sensitize dopamine neurons. To test this hypothesis, we expressed an excitatory DREADD in ventral tegmental area (VTA) dopamine neurons of female and male rats. We then administered clozapine-n-oxide (CNO) prior to each daily test session of the cued or uncued rat gambling task (rGT), in which rats try to maximise sugar pellet wins by responding for probabilistic rewards. The win-paired audiovisual cues of the cued rGT, inspired by the sound and lights of commercial gambling, promote risk taking. We found that chronic chemogenetic stimulation of VTA dopamine neurons resulted in suboptimal choice patterns on the cued rGT in both sexes, yet this altered choice behaviour was not observed on the uncued task. DREADD-mediated excitation of VTA dopamine selectively increased impulsivity in females, and drove rats of both sexes to subsequently self-administered more cocaine. However, contrary to our expectations, repeated dopamine neuron stimulation prevented cocaine-induced decision making deficits. In separate animals, we confirmed that chronic activation of the DREADD in VTA dopamine neurons led to locomotor sensitization, and increased electrophysiological markers of phasic, but not tonic, activity. These data confirm that a hyper-excitable dopamine system can both promote cognitive behaviours permissive of cocaine-taking as well as cocaine-taking itself, and that the presence of win-paired cues mediates the ability of sensitized midbrain dopamine to promote risk preference.

3-A-7 Neural bases of Decision making: Reinforcement, Variability and Exploration in Choice behavior

Maxime Come[1], Elise Bousseyrol[1], Steve Didienne[1], Marwen Belkaïd[2], Tarek Ahmed Yahia[1], Philippe Faure[1]

[1]ESPCI - Brain Plasticity Lab, [2]Istituto Italiano di Tecnologia

Objective: Behaving in an unusual, variable, or unpredictable manner is a fundamental ability, observed even when animals repeatedly face the same situation, for instance in decision-making settings (exploration). Yet, the neural bases underlying such behavior are still poorly understood. The dopaminergic (DA) system encodes rewards value, reinforces the best options and makes us choose them more frequently (exploitation). This work aims to assess DA dynamics in exploratory goal-directed decisions. Methods: We designed a conditioning task where mice learn to perform sequences of binary choices to obtain rewards. Animals are rewarded for non-repetitive choice sequences (when they choose the target leading to higher sequence complexity). Neural correlates of choice strategy are assessed using tetrodes recordings in the Ventral Tegmental Area (VTA) and fiber photometry (DA sensor in the Nucleus Accumbens (NAc)) during the task. Results: We show that mice progressively increase their choice variability and use a random-like choice strategy (Belkaïd et al, 2020), which seems to rely on DA dynamics. Indeed, when rewards are certain (all choices are rewarded), VTA DA neurons activity encode next choice's value, but this encoding is impaired in our complexity rule. Moreover, electrophysiological recordings suggest an adaptive modulation of DA neurons activity during the task, while DA release in the NAc displays continuous computations of Reward Prediction Errors (RPE) to adapt choice strategy online. Conclusion: We show evidence of mice ability to generate and reinforce a random-like choice strategy. Preliminary results suggest the involvement of meso-limbic DA signaling in this exploratory strategy.

3-A-8 Aversive stimulus coding revisited: brain state-dependent responses of reward and anty-reward systems to electrical footshocks

Gabriela Izowit[1], Martyna Marzec[1], Magdalena Walczak[1], Gniewosz Drwiega[1], Zuzanna Stawicka[1], Wojciech Solecki[1], Tomasz Blasiak[1]
[1]Jagiellonian University

Dopaminergic (DA) neurons of the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) are known for, among other functions, coding aversive information. One of the major VTA/SNc inputs relying information about aversive events and cues comes from lateral habenula (LHb). In both VTA/SNc and LHb two neuronal populations have been distinguished: value- and salience-coding. This division is based on the type of observed responses (excitatory or inhibitory) to aversive stimuli (AS). Given that basal activity of VTA/SNc DA neurons depends on alternating brain states under urethane anaesthesia, in the first set of experiments we checked whether VTA/SNc DA neurons responses to AS are also brain state-dependent. Secondly, we wanted to examine if basal activity of LHb neurons undergoes similar changes to those observed in VTA/SNc and if LHb responses to AS differ between two brain states observed under urethane. Firstly, we carried out in vivo extracellular recordings of single midbrain DA neurons combined with optotagging and recorded their responses to electrical footshocks from urethane anaesthetized rats. Secondly, we recorded activity and responses to AS of LHb neurons using Multi-Electrode Arrays. Consistently with literature, we observed two neuronal subpopulations of both VTA/SNc and LHb - excited and inhibited by AS. However, we also recorded previously undescribed populations of VTA/SNc and LHb neurons that are characterized by dynamic changes in the type of response to AS between low theta-frequency and slow wave brain states. This study sheds new light on interplay of LHb-->VTA/SNc in AS-coding as well as influence that general state of the brain may exert on processing of aversion. Funding: National Science Centre, PRELUDIUM 2019/33/N/NZ4/03011.

3-A-9 Cholinergic dysfunction in the dorsal striatum promotes habit formation and maladaptive eating

Mathieu Favier[1], Helena Janickova[2], Damian Justo[3], Ornela Kljackic[2], Léonie Runtz[1], Joman Natsheh[4], Tharick Pascoal[1], Jurgen Germann[1], Daniel Gallino[1], Jun-Il Kang[1], Xiang Qi Meng[1], Christina Antinora[1], Sanda Raulic[2], Jacob Jacobsen[5], Luc Moquin[1], Erika V
[1]Douglas Mental Health University Institute, McGill University, [2]Robarts Research Institute, University of Western Ontario, [3]GHU Paris Psychiatrie et Neurosciences (CMME, Hospital Sainte-Anne), INSERM UMR1266, [4]Palestinian Neuroscience Initiative, Al-Quds

Dysregulation of habit formation has been recently proposed as pivotal to eating disorders. Here, we report that a subset of patients suffering from restrictive anorexia nervosa have enhanced habit formation compared with healthy controls. Habit formation is modulated by striatal cholinergic interneurons. These interneurons express vesicular transporters for acetylcholine (VAChT) and glutamate (VGLUT3) and use acetylcholine/glutamate cotransmission to regulate striatal functions. Using mice with genetically silenced VAChT (VAChT conditional KO, VAChTcKO) or VGLUT3 (VGLUT3cKO), we investigated the roles that acetylcholine and glutamate released by cholinergic interneurons play in habit formation and maladaptive eating. Silencing glutamate favored goal-directed behaviors and had no impact on eating behavior. In contrast, VAChTcKO mice were more prone to habits and maladaptive eating. Specific deletion of VAChT in the dorsomedial striatum of adult mice was sufficient to phenocopy maladaptive eating behaviors of VAChTcKO mice. Interestingly, VAChTcKO mice had reduced dopamine release in the dorsomedial striatum but not in the dorsolateral striatum. The dysfunctional eating behavior of VAChTcKO mice was alleviated by donepezil and by l-DOPA, confirming an acetylcholine/dopamine deficit. Our study reveals that loss of acetylcholine leads to a dopamine

imbalance in striatal compartments, thereby promoting habits and vulnerability to maladaptive eating in mice.

3-A-10 Modeling treatments for effort-related motivational dysfunction: Assessment of novel atypical dopamine transport inhibitors

Alev Ecevitoglu[1], Nicolette Meka[1], Gayle Edelstein[1], Sonya Srinath[1], Renee Rotolo[1], Carla Carratala-Ros[2], Rose Presby[1], Jianjing Cao[3], Amarchi Okorom[3], Amy Newman[3], Merce Correa[2], John Salamone[1]
[1]University of Connecticut, [2]Universitat Jaume I, [3]NIDA-Intramural Research Program

People with depression, schizophrenia, Parkinsonism and other disorders have motivational dysfunctions such as anergia, avolition, and fatigue, which are relatively treatment-resistant. Animal models have been established to measure effort-related motivational dysfunction for developing new treatments. In studies of effort-based choice, rats are given the option of high effort/high reward vs low effort/low reward activities. By using the vesicular monoamine transport-2 inhibitor tetrabenazine (TBZ), a low effort bias that is thought to mimic motivational dysfunction in humans can be induced in rodents. Preclinical data indicate that these deficits can be reversed by dopamine transport (DAT) inhibitors, including amphetamines and methylphenidate, but not by drugs that selectively increase serotonin or norepinephrine transmission. Although classical DAT blockers can produce undesirable effects such as abuse liability, not all DAT inhibitors have the same neurochemical profile, and novel compounds with atypical binding characteristics are being developed. Ongoing studies are characterizing the effort-related effects of novel DAT inhibitors that are modafinil analogs with a range of binding profiles (JJC8-088, JJC8-089, and JJC8-091). JJC8-088 and JJC8-089 significantly increased selection of high effort responding by reversing the lever pressing suppression induced by TBZ. JJC8-088 (cocaine-like profile) also increased selection of high-effort progressive ratio responding. However, JJC8-091 failed to produce these outcomes, potentially due to its unique DAT binding profile. These findings and the continuing study of modafinil analogs may help identify novel therapeutics for effort-related aspects of motivational dysfunction observed in numerous psychopathologies.

3-A-11 Mechanistic Insight into Microbial Regulation of Psychostimulant Abuse

Samuel Mabry[1], Xixi Cao[2], Shalinkumar Patel[1], Anna Elam[1], David Saleeby[1], Hui Wu[2], Aurelio Galli[1], Angela Carter[1]
[1]University of Alabama at Birmingham, [2]Oregon Health and Science University

The abuse potential and psychomotor stimulant properties of amphetamines (AMPHs) have been associated with their ability to increase extracellular dopamine (DA) levels. This increase is mediated, at least in part, by the reversal of DA transporter (DAT) function, which causes non-vesicular DA release (DA efflux). Recent studies suggest that imbalances in the gut microbiome participate in the pathogenesis of substance use disorders. Microbial products such as short-chain fatty acids (SCFAs), are suspected to play a fundamental role in this process. Among SCFAs, butyrate is known to cross the blood-brain barrier and directly act on neurons and glial cells. Fusobacterium nucleatum (F. nucleatum) is a bacterial species that secretes butyrate and whose abundance is increased by AMPH abuse in both rodents and humans. It is important to note that butyrate is a potent inhibitor of histone deacetylases (HDACs) and that inhibition of HDACs robustly increases expression of both DAT mRNA and protein levels. Here, we report that colonization of the intestinal tract of gnotobiotic Drosophila with F. nucleatum significantly enhances AMPH-induced DA efflux and associated behaviors. This potentiation of AMPH actions by F. nucleatum was paralleled by oral administration of butyrate. Further, both

pharmacological inhibition and genetic knockdown of HDAC1 increased AMPH-induced DA efflux and locomotion as well as DAT expression. These data demonstrate that F. nucleatum modulates AMPH-induced behaviors through secretion of butyrate, inhibition of HDACs, elevation of DAT expression, and increased DA efflux. These findings suggest modulation of the gut microbiome, or their downstream targets, as a therapeutic approach for substance use disorders.

3-A-12 Exploring regulation and function of dopamine D3 receptors in alcohol use disorder. A PET [11 C]-(+)-PHNO study

Bernard Le Foll[1], Chidera Chukwueke[1], Christina Nona[1], Matthew McPHee[1], Esmaeil Mansouri[1], Dafna Rubin-Kahana[1], Diana Martinez[2], Christian Hendershot[1], Isabelle Boileau[1]
[1]University of Toronto, [2]Columbia University

Preclinical studies support an important role of dopamine D3 receptors (DRD3s) in alcohol use disorder (AUD). In animals, voluntary alcohol consumption increases DRD3 expression, and pharmacological blockade of DRD3s attenuates alcohol self-administration and reinstatement of alcohol seeking. However, these findings have yet to be translated in humans. This study used positron emission tomography (PET) and [11C]-(+)-PHNO to compare receptor levels in several dopamine D2 receptor (DRD2) and DRD3 regions of interest between AUD subjects in early abstinence (n = 17; 6.59 ± 4.14 days of abstinence) and healthy controls (n = 18). We recruited non-treatment seeking subjects meeting DSM-5 criteria for AUD. We examined the relationship between DRD2/3 levels and both alcohol craving and alcohol motivation/wanting, using a cue reactivity procedure and an intravenous alcohol self-administration (IVASA) paradigm, respectively. [11C]-(+)-PHNO binding levels in AUD subjects were significantly lower than binding in HCs when looking at all DRD2/3 ROIs jointly (Wilk's Ë = .58, F(6,28) =3.33, p = 0.013, ç2p = 0.42), however there were no region-specific differences. Binding values demonstrate -12.3% and -16.1% lower [11C]-(+)-PHNO binding in the SMST and SN respectively, though these differences did not withstand Bonferroni corrections. There was a positive association between [11C]-(+)-PHNO binding in the SN (almost exclusively reflective of DRD3) and alpha (lower values reflect higher alcohol demand) in the APT after Bonferroni corrections (r = 0.66, p = 0.0080). This demonstrates that AUD subjects with lower DRD3 levels in the SN exhibit increased demand for alcohol. Furthermore, the finding that binding in the SN is associated with alcohol demand warrants further examination.

3-A-13 Role of neuromedin S-expressing ventral tegmental area neurons in morphine behavior

Cristina Rivera-Quiles[1], Milagros Alday[1], Olivia Dodson[1], Michelle Mazei-Robison[1]
[1]Michigan State University

Opioid dependence and addiction constitute a major burden, but our limited understanding of the underlying neurobiology limits better interventions. Alteration in the activity and output of dopamine (DA) neurons in the ventral tegmental area (VTA) is known to contribute to effects, but the mechanisms underlying changes in VTA DA function remain unexplored. We used TRAP to identify gene expression changes in VTA DA neurons following chronic morphine and found that Neuromedin S (NMS) is enriched in VTA DA neurons, and its expression is robustly increased by morphine. However, whether all VTA DA neurons express NMS, and their potential functional impact has yet to be determined. Male and female NMS-Cre mice and wild-type littermates were used with Cre-dependent viral vectors to allow for DREADD-mediated activation (Dq) or inhibition (Di) of VTA-NMS neurons. We found that a subset of VTA DA neurons express NMS (<5%) and that they exhibit diverse projection targets, including the nucleus

accumbens. Using DREADDs, we found that activation (Dq,) or inhibition (Di,) of VTA-NMS neurons did not affect general locomotor activity or elicit CNO-conditioned place preference or aversion. We find that Dq mice exhibit increased morphine-induced locomotor activity and increased response to a challenge morphine + CNO injection. In contrast, Di mice show a trend for the opposite effect, with decreased morphine-elicited locomotor activity and decreased response to challenge. Ongoing studies are determining whether VTA-NMS neuronal activity modulates morphine conditioned place preference. Our current data suggest that VTA-NMS neurons represent a subset of VTA neurons that may be functionally relevant for morphine responses.

3-A-15 Dopaminergic circuit for compulsive eating behavior

Bokyeong Kim[1], Ja-Hyun Baik[1]
[1]Korea University

Dopamine serves a central role in motivated behavior and reward processing, in which dopamine D2 receptor (D2R) is intimately involved. Palatable food drives hedonic food consumption, and hedonic drive to feed is a key contributor to compulsive eating resulting in obesity. Reduction of striatal D2R availability is observed in obese patients. The similar deficit is also detected in drug addicts, suggesting D2R is important to compulsive behavior towards the reward. We observed that D2R knockout (D2R -/-) mice consumed significantly higher amount of palatable food when limiting the access to palatable foods. In the light/dark box test, D2R -/- mice showed increased palatable food consumption in the aversive context, displaying compulsive eating behavior. It has recently been reported that the central nucleus of the amygdala (CeA) is involved in orexigenic/anorexigenic feeding behavior related to a rewards system. We previously identified D2R (+) neurons from the CeA to the bed nucleus of the stria terminalis (BNST) as a dopaminergic circuit regulating impulsivity. Selective optogenetic activation of D2R (+) neurons in the CeA→BNST circuit attenuates palatable food consumption in light/dark box test. Conversely, optogenetic inhibition increases obsessive palatable food intake. Together, these data provide evidence that D2R (+) neurons in the CeA→BNST circuit can modulate compulsive eating behavior and may be a potential therapeutic target for obesity and eating disorders. [This work was supported by the Bio & Medical Technology Development Program Grant no: 2016M3A9D5A01952412]

D – Dopamine, Parkinson's Disease and neurodegeneration

3-D-16 Transient elevated dopamine pretreatment alleviates motor impairments in a 6-OHDA model of Parkinson's disease

Joe Brague[1], Rebecca Seal[1]
[1]University of Pittsburgh

Dopaminergic signaling is critically important to the regulation of motor functions in the mammalian nervous system. For example, the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpC) leads to severe motor deficits, as seen in patients with Parkinson's Disease (PD). Thus, novel strategies to treat the motor impairments of PD are still needed. Our finding that vesicular glutamate transporter 3 knockout (KO) mice do not develop motor impairments in a 6-OHDA mouse model of PD provide a unique opportunity to investigate the mechanistic basis for motor symptoms. KO mice show a circadian-dependent hyperdopaminergia, hyper locomotion, and upregulation of immature dendritic spines on dopamine 1 receptor containing medium spiny neurons (D1R MSNs). Unexpectedly, DA depleted KO mice exhibit an increase of mature D1R MSN spines and do not show motor impairments. I

hypothesize that transient elevated dopamine (TED) and subsequent DA depletion impart the spine and normalized behavioral phenotypes we see in DA depleted KO mice. Here I present preliminary data using designer receptors that support this hypothesis. The data indicate that TED mice show increased immature spines on D1R MSNs (n=11, $p<0.05$) and hyperlocomotive behavior (n=7, $P<0.01$) prior to depletion. Following depletion, TED mice exhibit an increase of mature spines on D1R MSNs (n=11, $p<0.01$) and normalized motor function (n=13, $p<0.001$). These data suggest that TED, prior to depletion, provides a potential therapeutic effect in PD by altering dendritic spines on D1R MSNs.

3-D-17 Expression of the synaptic vesicle glycoprotein 2C (SV2C) mediates dopaminergic neurotoxicity

Meghan Bucher[1], Faith Anderson[1], Joshua Bradner[1], Amy Dunn[2], Kristen Stout[3], Gary Miller[1]
[1]Columbia University, [2]The Jackson Laboratory, [3]Emory University

The vesicular handling of dopamine (DA) is dysregulated in Parkinson's disease (PD), resulting in an accumulation of cytosolic DA that has the potential to participate in neurotoxic reactions. To minimize the cytosolic pool of DA, the vesicular monoamine transporter 2 (VMAT2) sequesters DA within synaptic vesicles, and we hypothesize that the synaptic vesicle glycoprotein 2C (SV2C) plays a key role in retaining DA within synaptic vesicles. Here, we report an in vitro system using HEK293 cells to characterize the role of SV2C on vesicular DA dynamics and investigate the protective potential of SV2C against cytotoxicity induced by the DA neurotoxin MPP+. Using this system, we have demonstrated enhanced vesicular uptake of the VMAT2 substrate fluorescent false neurotransmitter 206 (FFN206) (24.8% increase) mediated by SV2C and SV2C mediated protection against the loss of FFN206 fluorescence induced by VMAT2 inhibitor tetrabenazine (60.4% slower decrease). We are now characterizing the effect of SV2C expression on MPP+ toxicity in vitro, and in complementary in vivo studies, SV2C-KO mice demonstrated a significant increase in MPTP vulnerability compared to WT. At the dose of MPP+ used, WT animals showed no significant neuronal loss in the substantia nigra determined by unbiased stereology compared to a 36.0% loss in SV2C-KO mice. Ongoing studies include 3DA and 3MPP+ uptake and leak assays in vesicles isolated from WT and SV2C-KO mice and HEK293 cells lacking or expressing SV2C, and MPP+ neurotoxicity assays in C. elegans expressing human SV2C to determine its effect on neuronal integrity and DA-mediated behaviors following MPP+ insult. Overall, these data identify SV2C as a novel mediator of DA cell vulnerability to endogenous and exogenous neurotoxicants.

3-D-18 CDK14 regulates alpha-synuclein levels and toxicity in Parkinson's Disease

Jean-Louis Parmasad[1], Konrad Ricke[1], Morgan Stykel[2], Brodie Buchner-Duby[2], Eric Lian[3], Nathalie Lengacher[3], Haley Geertsma[1], Steve Callaghan[1], Julianna Tomlinson[3], Michael Schlossmacher[3], William Stanford[3], Patrik Brundin[4], Scott Ryan[2], Maxime Rousseaux[1]
[1]University of Ottawa, [2]University of Guelph, [3]Ottawa Hospital Research Institute, [4]Van Andel Institute

Parkinson's disease (PD) is a debilitating and incurable neurodegenerative disease characterized by the abnormal accumulation of alpha-synuclein (α-syn), leading to the death of dopaminergic neurons in the substantia nigra. Patients with multiplications of the α-syn gene (SNCA) develop familial PD and animal models that overexpress α-syn replicate several features of PD. Thus, there is a clear link between disease pathology and α-syn levels, indicating the important role of α-syn in the manifestation of PD. Therefore, decreasing α-syn levels may be an effective approach to attenuate neurodegeneration in PD patients. We previously identified CDK14, a brain-abundant protein kinase, as a strong regulator of α-syn levels. In this study, we genetically reduced CDK14 levels in two mouse models of PD to assess the

impact of CDK14 as a therapeutic target. Our data suggest that CDK14 reduction improves bradykinesia and constipation in the PD mice, as well as lowers α-syn levels. Furthermore, reducing CDK14 levels was sufficient to limit the propagation of toxic α-syn preformed fibrils (PFFs) throughout the mouse brain as well as improved its associated phenotypes. Next, we pharmacologically inhibited CDK14 in human neurons derived from embryonic stem cells and observed robust reduction of α-syn levels. We then stressed mouse primary neurons with α-syn PFFs, followed by CDK14 inhibitor treatment, which attenuated the formation of α-syn oligomers. Finally, we performed CRISPR-Cas9-mediated knockdown of CDK14 in patient-derived neurons and found that this was sufficient to decrease pathological pSer129 α-syn accumulation. In summary, our findings point to CDK14 as an effective therapeutic target capable of decreasing α-syn levels and limiting its toxic effects.

3-D-19 Axonal domain structure and function as a key cell-autonomous characteristic of selective vulnerability in Parkinson's disease: a murine study of primary cultured neurons

Samuel Burke[1], Marie-Josée Bourque[1], Louis-Eric Trudeau[1]
[1]Université de Montréal

A current hypothesis suggests that vulnerable neurons in Parkinson's disease share common morphological characteristics including projecting to voluminous territories and having very long and highly branched axonal domains with very large numbers of neurotransmitter release sites. In this study, we used an in vitro culture system to compare the axonal domain of neuronal populations suspected to be vulnerable in PD to that of neuronal population considered at a lesser risk. In the first group, we included DA neurons of the SNc, noradrenergic neurons of the locus coeruleus (LC), serotonin neurons of the raphe nuclei (R), and cholinergic (ChAT+) neurons of the dorsal motor nucleus of the vagus (DMV). In the second group, we included DA neurons of the VTA, cholinergic neurons of the hypoglossal nucleus (XII), and cholinergic interneurons of the dorsal striatum (STR). Validating their differential vulnerability, we find that, when compared to PD-resilient neurons, a larger proportion of PD-vulnerable neurons degenerate in response to cell stress induced by hydrogen peroxide, despite displaying similar levels of reactive oxygen species production in mitochondria. We also find that they are endowed with larger axonal domains, that are more complex, have more axonal varicosities and with a higher proportion of varicosities that are positive for synaptotagmin 1. Globally, these findings support the hypothesis that axonal domain structure and function is a key cell-autonomous characteristic of selective vulnerability in PD.

3-D-20 Determining the role of retinoic acid on dopamine neurons selective vulnerability in a mouse model of Parkinson?s disease

George Sung[1], Jean-Francois Poulin[1]
[1]McGill University

Since discovering L-DOPA to treat Parkinson's disease (PD) in the 1960s, there are still no medication available to slow down PD. PD is the second most common neurodegenerative disease and is defined by motor symptoms, Lewy body (LB) propagation, and the loss of dopaminergic (DA) neurons. Several lines of evidence show that DA neurons expressing Aldh1a1 appear to be more vulnerable than neurons that don't express it, suggesting potential molecular determinants of selective vulnerability. Aldh1a1 confers neurons the ability to synthesize retinoic acid (RA), a multipurpose signaling molecule. Aldh1a1 is exclusively expressed in a subset of DA neurons, and the presence of Aldh1a1 in axonal terminals is responsible for the dorsal striatum having one of the highest levels of RA in the adult brain. We

generated a Flp-dependent viral vector overexpressing alpha-synuclein (aSyn), a protein present in LB and associated with familial cases of PD, in order to study selective vulnerability in mice. We observed a progressive loss of DA neurons at various timepoints after injection and we are characterising the vulnerability of Aldh1a1-expressing DA neurons. To assess the role of excess RA in DA neuron's degeneration, we will overexpress Aldh1a1 along with aSyn and expect RA precipitating cell loss. Our model in conjunction with other intersectional genetic tools will allow us to observe changes in cellular features of different DA neuron subtypes leading up to degeneration.

3-D-21 Investigating the role of alpha-synuclein in presynaptic microtubule dynamics in dopaminergic neurons

Alessandro Comincini[1], Samanta Mazzetti[2], Alessandra Calogero[3], Julie Parato[1], David Sulzer[1], Graziella Cappelletti[3], Francesca Bartolini[1]
[1]Columbia University, [2]fondazione grigioni per morbo di Parkinson, [3]Università degli Studi di Milano

α-synuclein, a protein involved in Parkinson's Disease, was shown to be physiologically implicated in dopamine release through an ill-defined mechanism. In disease states, α-synuclein loses its function by switching to a pathological prone-to-aggregate β-sheet conformation thus aggregating into toxic oligomers and fibrillar structures which ultimately can cause the death of the dopaminergic neurons of the substantia nigra pars compacta. Microtubules (MTs) are polymers composed of regulated α- and β-tubulin subunit assembly. In neurons dynamic MTs are a special pool of MTs with essential roles in both pre- and postsynaptic activity. Our in vitro findings indicate that α-synuclein may regulate dynamic MT assembly by working as a "dynamase". I investigated if α-synuclein colocalizes with the dynamic MT plus end binding protein EB3 and α- or γ-tubulin by proximity ligation assays in human and mouse brain and, in differentiated PC12 cells overexpressing α-synuclein. I found that α-synuclein and α-tubulin directly associate in the healthy human brain, and that this interaction is mostly localized in the presynaptic compartment in the mouse corpus striatum. A high degree of colocalization was also seen for EB3, γ-tubulin and tyrosinated α-tubulin, a marker of dynamic MTs. These data together support the idea that α-synuclein may act as a presynaptic MT "dynamase" at sites of dopamine release. To explore this possibility, I am currently investigating whether α-synuclein is a bona fide "dynamase" in primary neurons and if it regulates presynaptic MT nucleation at en passant boutons using live-cell imaging of presynaptic markers in WT and α-synuclein KO dopaminergic neurons isolated from ventral midbrain.

3-D-23 A novel target for neuroprotection: The small GTPase Rin inhibits LRRK2 to promote autophagy and reduce alpha-synuclein pathology

Anne-Marie Castonguay[1], Julia Obergasteiger[1], Mattia Volta[2], Martin Lévesque[1]
[1]Laval University, [2]Eurac Research

Parkinson's disease (PD) is characterized by the accumulation of misfolded alpha-synuclein (aSyn) in the substantia nigra (SNc), leading to the death of dopaminergic (DA) neurons. The mechanisms underlying aSyn pathology are still unclear but hypothesized to involve autophagy and endosome-lysosome pathways (ALP). LRRK2 mutations are a major cause of familial and sporadic PD. Pharmacological inhibition of LRRK2 kinase activity ameliorates ALP deficits and reduces pS129-aSyn inclusions, indicating that these phenotypes depend on LRRK2 hyperactivation. We observed selective down-regulation of the novel PD risk factor RIT2 in LRRK2 mutant cells (G2019S). RIT2 encodes the small GTPase Rin, which is enriched in DA neurons and reduced in the SNc of PD brains. We aim to evaluate if Rin can modulate LRRK2 kinase activity to rescue alterations in autophagy and promote aSyn clearance. Rin

overexpression in LRRK2-G2019S neuroblastoma cells rescued the alterations in ALP and diminished aSyn inclusions. In vivo, viral mediated overexpression of Rin prevented motor deficits induced by AAV-A53T-aSyn injection. Overexpression of Rin also protected against the loss of dopaminergic axons in the striatum and neuronal degeneration in the SNc. Furthermore, RIT2 overexpression prevented the A53T-aSyn-dependent increase of LRRK2 kinase activity in vivo. On the other hand, reduction of RIT2 levels leads to defects in the ALP, similar to the ones induced by the G2019S LRRK2 mutation. Our data indicate that RIT2 is required for correct lysosome function, inhibits overactive LRRK2 to ameliorate ALP impairments and counteract aSyn aggregation and related deficits. Targeting RIT2 could thus represent a novel strategy to combat neuropathology in familial and idiopathic PD.

3-D-24 Age related autophagy impairments in directly reprogrammed dopaminergic neurons in patients with idiopathic Parkinson's disease

Janelle Drouin-Ouellet[1], Marcella Birtele[2], Shelby Shrigley[2], Fredrik Nilsson[2], Maria Pereira[2], Yogita Sharma[2], Romina Vuono[3], Thomas Stoker[3], Johan Jakobsson[2], Roger Barker[3], Karolina Pircs[2], Malin Parmar[2]

[1]University of Montreal, [2]Lund University, [3]University of Cambridge

Understanding the pathophysiology of Parkinson's disease (PD) has been hampered by the lack of models that recapitulate all the critical factors underlying its development. Using a novel and highly efficient approach, we generated functional induced dopaminergic neurons (iDANs) that were directly reprogrammed from dermal fibroblasts of patients with idiopathic PD (n=19) as well as sex- and age-matched healthy donor (n=10) to investigate whether such cells have deficits in autophagy. We show that iDANs derived from PD patients exhibit lower basal chaperone-mediated autophagy as compared to iDANs of healthy donors. Furthermore, stress-induced autophagy resulted in an accumulation of macroautophagic structures in induced neurons (iNs) derived from PD patients, independently of the specific neuronal subtype but dependent on the age of the donor. Finally, we showed that these impairments in autophagy in the iNs derived from idiopathic PD patients lead to an increase in phosphorylated alpha-synuclein, a hallmark of PD pathology. Taken together, our results show that direct neural reprogramming provides a patient-specific model to study neuronal features relevant to idiopathic PD.

3-D-25 The role of glutamate co-transmission by serotonin neurons of the dorsal raphe nucleus in the expression of L-Dopa-induced dyskinesia

Lydia Saïdi[1], Christophe Proulx[1], Martin Parent[2]

[1]Cervo, [2]Université Laval

Parkinson's disease is characterized by the progressive loss of midbrain dopaminergic neurons that innervate the striatum. The dopamine precursor L-3,4-dihydroxyphenylalanine (L-Dopa) is the most effective pharmacotherapy but its chronic use is hampered by adverse effects such as abnormal involuntary movements (AIMs), also termed L-Dopa-induced dyskinesia (LID). Recent studies have shown the crucial role of serotonin (5-HT) neurons in LID expression. Through this study, we specifically addressed the functional role of glutamate co-transmission by 5-HT neurons of the dorsal raphe nucleus (DRN) in the regulation of motor behavior and LID expression. We used CRIPSR-Cas9 technology and viral injections to knock-out or overexpress the atypical vesicular glutamate transporter 3 (VGluT3), specifically in 5-HT neurons of the DRN in adult mice. After extensive behavioral testing, these mice were injected with 6-OHDA in the medial forebrain bundle to selectively lesion DA axons, and then

treated with L-Dopa to induce AIMs. RT-qPCR assay, RNAscope and immunohistochemistry confirm the depletion or overexpression of VGluT3 in AAV-infected 5-HT neurons of the DRN. High-resolution confocal analysis of target sites reveals a decreased number of axon varicosities emitted by VGluT3-depleted 5-HT neurons. Before dopamine lesion and L-Dopa administration, VGluT3-depleted mice show increased spontaneous motor activity and impulsivity, as well as anhedonia. Compared to controls, VGluT3-depleted mice show lower motor disabilities induced by 6-OHDA and exacerbated AIMs caused by L-Dopa administration. VGluT3 that is co-released by 5-HT neurons of the DRN appears to be involved the regulation of spontaneous motor behaviors and impulsivity, as well as in the expression of LID and anhedonia.

3-D-26 Cerebroventricular microinjections of MPTP on adult zebrafish induces mitochondrial fragmentation in dopaminergic neurons, sensorimotor impairments and the activation of neural stem cells

Michael Kalyn[1], Marc Ekker[1]
[1]University of Ottawa

Mitochondria are dynamic organelles that mediate the energetic supply to cells and mitigate oxidative stress through the intricate balance of fission and fusion. Mitochondrial dysfunction is a prominent feature within Parkinson disease (PD) etiologies. To date, there have been conflicting studies of neurotoxin impact on dopaminergic cell death, mitochondrial function and behavioral impairment using adult zebrafish. Here, we performed cerebroventricular microinjections (CVMIs) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on adult transgenic zebrafish that resulted in significant reductions in the number of dopaminergic neurons within the telencephalon and olfactory bulbs (OB) of Tg(dat:eGFP) fish. Visualization of mCherry and mitochondrial gene expression analysis in Tg(dat:tom20 MLS:mCherry) fish reveal that MPTP induces mitochondrial fragmentation in dopaminergic neurons and the activation of the pink1/parkin pathway involved mitophagy. This loss of dopaminergic neurons translated into a transient locomotor and olfactory phenotype. Moreover, treatment with bromodeoxyuridine (BrdU) and the assessment of markers associated with neurogenesis and neural stem cell proliferation reveal an immediate proliferative response in MPTP-injected zebrafish. Taken together, these data can contribute to a better understanding of the mitochondrial impact on dopaminergic survivability, as well as, aid in identifying regulators of dopaminergic neuronal regeneration. Supported by the Natural Sciences and Engineering Research Council of Canada.

E – Development and diversity of the dopamine systems

3-E-27 Adolescent nicotine exposure disrupts its anxiogenic properties in adulthood

Lauren Reynolds[1], Sophie Fayad[1], Claire Nguyen[1], Thomas Topilko[2], Aylin Gulmez[1], Fabio Marti[1], Nicolas Heck[3], Nicolas Renier[2], Alexandre Mourot[1], Philippe Faure[1]
[1]ESPCI Paris, [2]ICM Institut du Cerveau et de la Moelle Epinière, [3]Sorbonne Université

Dopamine (DA) circuity is increasingly considered as a "plasticity system" where its structure and function is shaped by experience during development, creating adaptive behavioral profiles that can endure throughout the lifetime. This plasticity may also demarcate a period of increased vulnerability to environmental insult: adolescent initiation of drug use, in particular, is associated with an increased risk of psychopathologies linked with DA dysfunction. This may be especially true for nicotine, as up to 90% of adult smokers began as adolescents. Nicotine acts on distinct DA pathways to produce both reinforcing and anxiogenic effects: the activation of nucleus accumbens (NAc)-projecting DA neurons

produces reinforcement, whereas the simultaneous inhibition of amygdala (AMG)-projecting DA neurons produces anxiety-like behavior. How experience with nicotine in adolescence modulates the anxiogenic effects of later re-exposure is largely unexplored. Remarkably, we found that the anxiogenic response to nicotine injection is abolished in adult mice pre-exposed to nicotine in adolescence, but not those pre-exposed in adulthood. Using iDISCO and ClearMap, we found that adolescent nicotine exposure augments brain-wide activation in response to an acute nicotine injection in adulthood, with notably increased cFos+ neurons in the NAc and AMG. Using single-unit recordings we found that nicotine-induced activation was stronger in adult mice exposed to nicotine in adolescence, nicotine-induced inhibition, however, was unchanged. Together, our results highlight how diverse DA pathways can be impacted by experience in adolescence, and further suggest that developmentally induced "imbalance" of these pathways may alter vulnerability profiles for later DA-dependent psychopathologies.

3-E-28 Dopamine synaptogenesis in a mouse model of autism spectrum disorder

Sarah Martin[1], Valentine Greffion[1], Jean-Francois Poulin[1]
[1]McGill University

Autism Spectrum disorder (ASD) is a diverse neurodevelopmental condition with certain behaviours that are common across the spectrum, such as challenges with social interactions and repetitive behaviours. Abnormal formation of dopamine (DA) circuits is hypothesized to underlie some aspects of these characteristic behaviours, and research in this area is ongoing. However, DA synaptogenesis in normal development is still poorly understood. Using intersectional genetics, we are investigating the neurobiological basis of ASD by mapping the normal development of DA circuits at critical timepoints (P0, P7, P14, P21, P28) and comparing this to a mouse model of ASD (Shank3b knock-out). We are using mice that express Cre and Flp recombinases to control the expression of synaptophysin-GFP (Syn-GFP) in DA neurons with a genetic reporter (RC::FPSiT) or a virus injection. Since synaptophysin is located on neurotransmitter vesicles, the expression of Syn-GFP allows us to visualize DA release sites in the striatum with high-resolution microscopy. We are also currently investigating the activation of DA circuits in the ventral striatum during social behaviour using in vivo calcium imaging. We hypothesize that there is less DA synaptogenesis in the ventral striatum in Shank3b knock-out mice compared to wildtype mice, resulting in lower DA circuit activity in the ventral striatum during social interaction compared to wildtype mice. This series of experiments will provide insights on structural and functional features of DA circuits, and how they are altered in a model of ASD.

3-E-29 Netrin-1 regulates GABAergic neuronal migration and Substantia nigra development in the ventral midbrain

Divya D. A. Raj[1], Sara Brignani[1], Ewoud Schmidt[1], Ozge Dudukcu[1], Laurens Grossouw[1], Youri Adolfs[1], Alain Chedotal[2], Jeroen Pasterkamp[1]
[1]Utrecht University, [2]Sorbonne Université

Cell migration in the central nervous system is known to be regulated by guidance cues produced locally by cells residing at choice points. We show a hitherto unknown role for Netrin-1 in the positioning of dopaminergic and GABAergic neurons in the ventral midbrain. Mice lacking Netrin-1 protein show increased lateral-ventral migration of dopaminergic neurons of the compacta (SNc). Normally, SNc neurons project their dendrites ventrally into the GABA-rich Substantia nigra pars reticulata (SNr). In Netrin-1 KO mice, GABAergic neurons of the anterior SNr fail to migrate ventrally to form this brain

nucleus. Intriguingly, while conditional ablation of Netrin-1 from local dopaminergic neurons in the midbrain affects positioning of GABAergic neurons within the VTA, it does not affect the formation of SNr. We find that Netrin-1 derived from forebrain neuronal axons in the cerebral peduncle affects migration and positioning of neurons in the SNc and SNr. We demonstrate how Netrin-1 from different cellular sources affect positioning of distinct GABA neurons in the ventral midbrain. This study signifies the role of axons as "carriers" of guidance cues to affect cellular organization in the developing brain.

G – Imaging Dopamine

3-G-30 Estimating absolute dopamine concentrations from biosensor data using Michaelis-Menten kinetics of the dopamine transporter

Aske Ejdrup[1], Jakob Dreyer[2], Leonie Posselt[1], Ulrik Gether[1]
[1]University of Copenhagen, [2]Lundbeck

Measurements of extracellular dopamine (DA) concentrations are vital to understand DA functions and the pathobiology of a host of disorders, such as Parkinson's, attention deficit hyperactivity disorder (ADHD) and schizophrenia. Microdialysis (MD) and fast-scan cyclic voltammetry (FSCV) have both contributed greatly but suffer different drawbacks, including temporal resolution, length of measurements and specificity. Recently developed genetically encoded biosensors allow for extended recordings at high resolution, but their output of light fluctuations have only been used to describe relative changes in arbitrary units. We propose a method to estimate absolute concentrations of DA using well established models of dopamine release and Michaelis-Menten kinetics of the dopamine transporter (DAT), the primary source of DA clearance. Using the dopamine sensor dLight1.3b we observed DA transients in the nucleus accumbens (NAc) in response to novel stimulus akin to FSCV measurements and tonic levels like those of multiple-cyclic square wave voltammetry and pharmacology studies. Additionally, exposure to cocaine yielded a relative rise, and Gi-induced hampering of VTA through the designer receptor hM4Di a relative fall, in basal levels of DA in NAc similar to reports from MD. The method yields robust results across mice and estimates a Vmax of DAT in accord with literature values. It requires only a short minutes-long trace to fit after initial photo-bleach has settled, after which pharmacological treatment that alter the DA system can be started. The method has the potential to be applied to other credibly modelled neurotransmitter systems with well-described uptake parameters

3-G-31 Normative values of neuromelanin-sensitive MRI signal in older adults obtained using a standard protocol for acquisition and analysis

Rami Al Haddad[1], Mira Chamoun[2], Christine Tardif[2], Serge Gauthier[2], Synthia Guimond[1], Clifford Cassidy[1]
[1]University of Ottawa, [2]McGill University

Background: Neuromelanin-sensitive MRI (NM-MRI), has become an increasingly popular method to measure integrity and function of the dopamine system in the human brain. NM is synthesized via iron-dependent oxidation of catecholamines and accumulates over the lifespan in the Substantia Nigra (SN) and Locus Coeruleus (LC). Consistent with this, age-related increase in NM-MRI signal has been reported. To advance the potential use of NM-MRI as a biomarker for neuropsychiatric disorders, it is necessary to establish the normative range of the NM signal in healthy individuals. Here we collected NM-MRI scans from 152 healthy participants over 53 years old and 33 healthy participants from 18-52 years old. We aimed to demonstrate the effect of age on SN NM-MRI signal and provide normative values of SN signal and volume for older adults. Methods: NM-MRI data were analyzed after being registered to standardized space. NM-MRI contrast-to-noise ratio (CNR) was calculated for all SN voxels

relative to a reference region in the crus cerebri. Voxelwise linear regression analysis of SN CNR was conducted with age as an independent variable. Results: There was a significant cluster of SN voxels showing increasing CNR with age (550 SN voxels, p corrected<0.05, permutation test); these voxels were located in the core of the SN. There was also a significant cluster of voxels showing decreasing CNR with age (556 SN voxels, p corrected<0.05); these voxels were on the SN periphery. The mean CNR in the group of older adults was 10.0%±1.48 for left SN and 10.2%±1.50 for right SN. Conclusion: These results confirm the necessity of considering age in NM-MRI data analyses and provide normative values of NM-MRI signal, which could help biomarker development efforts using this method in older adults.

3-G-32 Spatiotemporal topography of striatum-wide dopamine release to salient stimuli and during Pavlovian learning

Mai-Anh Vu[1], Michelle Wen[2], Eleanor Brown[1], Timothy Otchy[3], L. Nathan Perkins[4], David Boas[1], Mark Howe[1]

[1]Boston University, [2]Harvard University, [3]Meta, [4]Apple

Dopamine release in the striatum is critical for diverse functions, including motivation, reward response, motor control, learning, and memory. Recent studies have provided evidence that dopamine release to cues, rewards, and movements varies in amplitude and timing across striatal sub-regions, suggesting that region-specific dopamine signals may support distinct functions in learning and behavior. Current approaches have been limited to measuring dopamine release across only one or two small striatum regions in a given subject. Thus, a complete view of the spatiotemporal evolution of rapid dopamine signals during learning and behavior throughout the striatum is lacking. To address this, we have developed a multi-fiber photometry approach to monitor dopamine release with sub-millimeter spatial resolution at over 50 locations simultaneously throughout the striatum in awake, behaving mice expressing the fluorescent dopamine indicator dLight 1.3. Our chronic implants allow us to track changes in signaling on timescales ranging from 10s of milliseconds to weeks during learning. We recorded dopamine release throughout the striatum using this approach as mice were presented with salient stimuli and then as they were trained in a Pavlovian conditioning task. Preliminary data suggest that there are spatial variations across 3 dimensions in dopamine release to conditioned and unconditioned stimuli and rewards, and in the dynamics of the cue- and reward-related signals across Pavlovian learning. Taken together, these initial findings provide the largest scale description of rapid dopamine release topography in the striatum to date and define the spatial territories over which functional dopamine signals may influence distinct aspects of learning and behavior.

3-G-33 Neuromelanin-sensitive MRI correlates with danger and safety cues in human fear conditioning fMRI

Rami Hamati[1], Bianca Chidiac[1], Cecelia Shvetz[1], Clifford Cassidy[1], Lauri Tuominen[1]

[1]University of Ottawa

Dopamine (DA) activity in regions such as the striatum and frontal cortex signal fear (CS+) versus safety (CS-) cues during fear conditioning in rodents. However, the relationship between DA and cue-dependent learning has not been well established in humans. The objective of this study was to establish the relationship between DA function and fear conditioning in healthy humans. Specifically, we asked whether neuromelanin-sensitive magnetic resonance imaging (NM-MRI) of the substantia nigra (SN), a proxy of DAergic function, predicts CS+ or CS- blood-oxygen-level-dependent (BOLD) activity during fear conditioning functional MRI (fMRI). Nineteen healthy individuals underwent NM-MRI and

fMRI during a fear conditioning task to assess BOLD responses to CS+ associated with mild electrical stimulation and to CS- associated with the absence of a stimulus. We then employed a voxel-wise analysis of CS+ or CS- BOLD signal with NM-MRI signal in the SN and age as regressors. We found the NM-MRI signal in the SN correlated with CS+ or CS- BOLD activations contingent on whether a brain region processes fear or safety. A cluster of positive correlation between the NM-MRI signal and BOLD response in the left putamen to CS+, but not CS-, was found. In contrast, a cluster of positive correlation in the left lateral orbitofrontal cortex to CS-, but not CS+, was found. These preliminary findings were not corrected for multiple comparisons. NM-MRI signal in the SN may predict cue-dependent learning and may provide a framework for studying DA dysregulation during fear conditioning in psychiatric disorders.

3-G-34 Imaging genetically-encoded neurotransmitter sensors and neural activity using head-mountable miniscopes

Waylin Yu[1], Kevin Zitelli[1], Jonathan Zapata[1], Alice Stamatakis[1]
[1]Inscopix, Inc.

Genetically encoded dynamic fluorescent sensors, such as calcium and voltage sensors, are powerful tools for monitoring neural activity. Recently developed neurotransmitter sensors represent a promising addition to this family of sensors. To date, neurotransmitter sensors have been used for in vivo fiber photometry, which lacks spatial resolution, or 2-photon microscopy - which offers spatial resolution but requires head fixation, thus limiting free animal behavior. Miniscopes like the nVistaTM system have been used to image similar sensors such as GCaMP, allowing for free behavior while monitoring neural activity with high spatial resolution. Here, we demonstrate the feasibility of imaging neurotransmitter sensors during free behavior using Inscopix miniscopes. Mice were injected with the GPCR-activation-based dopamine sensor GRABDA in the dorsal striatum and with the opsin ChrimsonR in the substantia nigra. Using the optogenetically-enabled miniscope nVokeTM system, GRABDA activity was monitored. Dopamine release was elicited from nigral fibers via stimulation of ChrimsonR, mediating an increase in GRABDA signal in the dorsal striatum. In another experiment, amphetamine in mice led to an increase in GRABDA signal in the striatum. Finally, a red-shifted variant of GRABDA was injected into the dorsal striatum along with the green calcium sensor GCaMP. Using the dual-color imaging nVueTM system, an increase in dopamine signal was measured in response to amphetamine while GCaMP activity was simultaneously monitored at single-cell resolution. These findings show that miniscopes are an excellent tool for imaging genetically encoded neurotransmitter sensors, and pave the way forward for taking full advantage of such comprehensive imaging platforms.

I – Anatomy and physiology of Dopamine systems

3-I-37 Aging effects on the density of dopamine-glutamate neurons in the ventral midbrain

Jacquelyn Tomaio[1], Sixtine Fleury[1], Yoon Seok Kim[2], Lief Ericsson Fenno[2], Charu Ramakrishnan[2], Karl Deisseroth[2], Susana Mingote[1]
[1]City University of New York, [2]Stanford University

Dopamine (DA) neurons in the ventral midbrain play a critical role in motivation, motor and reinforcement learning, and memory. A subpopulation of DA neurons in the ventral midbrain expresses vesicular glutamate transporter 2 (VGLUT2) and can co-release glutamate (GLU). Fate-mapping experiments in mice have shown 100% of DA neurons co-express VGLUT2 (Poulin et al., 2018; Steinkellner et al., 2018) during early development. However, in adulthood, this distribution changes to

30% in the ventral tegmental area (VTA) and 10% in the substantia nigra pars compacta (SNc) (Mingote et al., 2019; Steinkellner et al., 2018). Thus, most DA neurons switch from a DA-GLU to a DA-only phenotype during development; however, how aging affects neurotransmitter switching in mice has not been addressed. To test this, we used INTRSECT 2.0 viral vectors in TH Flp; VGLUT2 Cre mutant mice to distinctively label two subpopulations of DA neurons. We labeled DA-only neurons (not expressing VGLUT2) with the fluorescent protein mCherry, and DA-GLU neurons with yellow fluorescent protein. We quantified the number of DA-GLU and DA-only neurons in the VTA and SNc in adult (3 months old), middle-aged (1-year-old), and old-aged (2 years old) mice. Our results showed a significant decrease in SNc DA-GLU neuron density in old-aged mice. Our preliminary in situ hybridization data also showed a significant reduction of total VGLUT2 transcripts in the VTA, and a trending decrease of VGLUT2 transcripts in DA-GLU neurons, with age. We did not observe these differences in the SNc. Since DA-GLU co-transmission is conserved through phylogeny and present in humans, our rodent research will convey new insights into how aging affects the DA system.

3-I-38 Striatal inputs coordinate oscillations in acetylcholine and dopamine

Anne Krok[1], Nicolas Tritsch[1]
[1]New York University

It is widely believed that there exists a steady state, basal tone of neuromodulators like ACh and DA that is periodically interrupted by phasic increases or decreases signaling salient events that need to be attended to, acted on or learned from. Here we show that extracellular ACh levels in the dorsal striatum of mice constantly undergo large fluctuations several times a second, even during quiet wakefulness. We show that these fluctuations are driven by coordinated spiking of cholinergic interneurons. These fluctuations grow larger in amplitude during movement, in phase with acceleration, and are comparable in time course and magnitude to phasic ACh responses typically associated with reward. Importantly, these ACh fluctuations maintain a specific relationship to striatal DA levels across behavioral contexts. These findings question the existence of a basal tone for ACh and DA, and suggest that phasic increases and decreases in ACh and DA are not uniquely associated with reward and salient behavioral events. Rather, our data indicate that striatal DA and ACh signaling is consistently and intrinsically structured, forcing a reconsideration of our understanding of modulation of neural activity and behavior by ACh and DA.

J- Dopamine and brain circuitry

3-J-38 Local dopaminergic modulation of the serotonergic raphe

Luca Nava[1], Yann Pelloux[2], Leonardo Bontempi[2], Andrea Locarno[2], Noemi Barsotti[3], Massimo Pasqualetti[3], Raffaella Tonini[2]
[1]Istituto Italiano di Tecnologia_Università degli Studi di Genova, [2]Istituto Italiano di Tecnologia, [3]Università degli Studi di Pisa

The main neuromodulatory serotonergic system in the brain includes the dorsal raphe nucleus (DRN) that in addition to serotonergic neurons (DRN5-HT) comprises dopaminergic (DA) cells (DRNDA). DRNDA neurons, which have been shown to respond to incentive stimuli and to modulate wakefulness, are anatomically placed close to DRN5-HT cells. This raises the possibility of crosstalk between the raphe serotonergic (5-HT) and dopaminergic (DA systems. Nevertheless, evidence of a functional interplay between DRNDA and DRN5-HT subpopulations within the DRN is still lacking. Here, we have been combining electrophysiology and optogenetic approaches ex-vivo together with fiber photometry in vivo

to investigate how DRNDA neurons modulate the activity of DRN5-HT cells, and thereby the release of 5-HT in subcortical DRN target regions (i.e., dorsal striatum - DS and central amygdala -CeA). We found that exogenous DA application in the DRN boosted the firing activity of DS- or CeA-projecting DRN5-HT neurons (DRN5-HT to DS; DRN5-HT to CeA). While this DA-mediated modulation was insensitive to dopamine D1 or D2 receptor antagonism, it was blocked by the application of the adrenaline α1 receptor (α1R) antagonist prazosin. We obtained similar results in tyrosine hydroxylase (TH)-CRE mice that were injected with an adeno-associated viral (AAV) vector encoding the opsin Chrimson in the DRN; light-activation of DRN TH-positive neurons resulted in the α1R-mediated increase of DRN5-HT to DS or DRN5-HT to CeA firing rate. By using genetically encoded sensors for monitoring the temporal dynamics of 5HT signal in the DS or CeA, we are currently assessing the impact of atypical DRN DA signaling through α1R on 5-HT release, which might be relevant for the modulation of reward-guided motor behavior.

3-J-39 Distinct physiological and supraphysiological effects of dopaminergic neurons on nucleus accumbens reward processing

Sotiris Masmanidis[1], Charltien Long[1], Kwang Lee[1], Long Yang[1]
[1]University of California Los Angeles

Striatal function is strongly regulated by midbrain dopaminergic (DA) neuron input. Some behavioral studies suggest that, in addition to regulating learning (i.e., plasticity), DA neurons are capable of rapidly influencing striatal spiking activity on short (subsecond to second) timescales. The temporal coincidence of reward-evoked DA signals with nucleus accumbens (NAc) spiking has led to the view that rapid DA signaling is a key driver of reward activity in NAc neurons. However, this hypothesis has not been rigorously tested. To address this, here we combine in vivo electrophysiology, fluorescence DA sensing, and optogenetics. We use silicon microprobes to record spiking activity from dozens of NAc neurons, and an integrated optical fiber to concurrently monitor local DA signaling with dLight. In parallel, we perform optogenetic manipulations of VTA DA neurons in DAT-Cre mice, to transiently raise or reduce DA levels in the NAc. We find that optogenetically evoked DA release has minimal effects on NAc firing rates unless the level of DA release is multiple-times higher than the level corresponding to reward delivery. We also find that optogenetic suppression of DA neurons has minimal effect on NAc firing rates during reward delivery. Taken together, these findings suggest that NAc neural responses to rewards on rapid timescales are largely uncoupled from physiological, but not supraphysiological DA activity. These results challenge the current dogma that DA neurons normally play an important role in rapidly modulating striatal activity to influence imminent or ongoing behaviors. Finally, they suggest a critical need for the field to distinguish between what DA neurons normally do in the brain, and what they are capable of doing under supraphysiological conditions.

3-J-40 D1 receptor mediated dopaminergic modulation of nucleus incertus to interpeduncular nucleus input - a possible neuronal mechanism for stress-induced novelty preference deficiencies

Agata Szlaga[1], Patryk Sambak[1], Andrew Gundlach[2], Anna Blasiak[1]
[1]Jagiellonian University, Krakow, Poland, [2]The University of Melbourne, Parkville, Australia

Appropriate novelty/familiarity discrimination is crucial for proper functioning. Many neuropsychiatric disorders manifest in atypical reactions to novelty and stress plays a key role in the development of this deficiency. Here we investigated the nucleus incertus (NI) to interpeduncular nucleus (IPN) innervation.

NI is a stress-sensitive brainstem nucleus, and directly innervates the IPN, a neuroanatomical substrate for familiarity signalling. Concomitantly, dopamine (DA) has an established role in motivation and novelty preference. Aim was to investigate the functional NI to IPN connectivity, along with interactions of this innervation and DA/D1R signalling at the level of IPN neurons. Whole-cell patch-clamp recordings of IPN activity were combined with optogenetic stimulation of NI-originating fibres and D1R agonist application. D1R agonist was applied during multielectrode array (MEA) ex vivo recordings. Viral vector-based retrograde tract-tracing was combined with immunofluorescent staining. Upon optogenetic stimulation, mostly light-evoked inhibitory postsynaptic currents (le iPSCs) were observed in the IPN. Among the neurons sensitive to optogenetic stimulation, 61.5% was excited by D1R activation and analysis of le iPSC shape showed that D1R activation decreased their amplitude. Tract-tracing revealed that the ventral tegmental area (VTA) is the main source of DA terminals in the IPN. MEA recordings revealed interaction of DA/D1R signalling in the IPN and VTA. These electrophysiological and anatomical data suggest a role of NI-IPN-VTA pathway in the control of novelty preference and attentional processes, with DA/D1R signalling diminishing the negative influence of brainstem-originating stress signals on novelty preference. Funding: UMO-2018/30/E/NZ4/00687

3-J-41 A reaction diffusion model of dopaminergic and cholinergic traveling waves in the striatum

Joshua Goldberg[1], Jeffery Wickens[2]
[1]The Hebrew University of Jerusalem, [2]Okinawa Institute of Science and Technology

The maintenance of a neurochemical balance between dopamine (DA) and acetylcholine (ACh) is widely believed to be necessary for normal striatal function. However, how this balance is dynamically orchestrated is not entirely understood. To address this question, we propose an activator-inhibitor reaction-diffusion model that uses sound assumptions about the morphology of cholinergic interneuron (CIN) and DA axons and about the local, physiological coupling between them. We find that the model gives rise to self-organized coupled traveling waves of DA and CIN activation, and spatial patterns of localized "hills of activity". This simplified model is at once compatible with three recent experimental observations: 1) the dissociation between the firing of midbrain DA neurons and striatal DA levels; 2) the demonstration that CINs can activate nicotinic ACh receptors on striatal DA fibers and elicit DA release; and 3) the observation of traveling waves of striatal DA and in the CIN neuropil. We propose that the intrinsic striatal circuitry is well-suited to orchestrate the ACh-DA balance independently of midbrain DA neuron activity, with the dynamical formation of functional neurochemical striatal compartments.

K – Dopamine receptors, transporters & signalling

3-K-42 Characterizing dopamine kinetics and uptake pharmacology in a novel mouse line expressing the A313V dopamine transporter mutation.

Conner Wallace[1], Ali Salahpour[2], Sara Jones[1]
[1]Wake Forest School of Medicine, [2]University of Toronto

Dopamine transporter (DAT) deficiency syndrome is a fatal disorder leading to progressive Parkinsonism and dystonia. Children who are afflicted often do not live through adolescence and there is no long-term treatment. This condition is caused by distinct point mutations that alter expression and function of the DAT. The objective of this study was to characterize baseline dopamine kinetics and DAT pharmacology in mice expressing the A313V DAT mutation. Mice (n = 12 wild type, n = 11 mutants) underwent

locomotor experiments followed by ex vivo fast scan cyclic voltammetry. Baseline recordings were taken in the dorsolateral striatum followed by treatment with amphetamine (0.1 - 10.0 µM) or cocaine (0.3 - 30.0 µM). We found that mutants had greater locomotion and larger evoked dopamine release. However, uptake was slowed by about 90% in the mutants. In wild type mice, cumulative cocaine produced an inverted U-shaped curve, initially increasing then reducing dopamine release, whereas release was dose-dependently reduced in mutants. Amphetamine reduced dopamine release in both groups, but this effect was blunted in mutants. Both cocaine and amphetamine slowed uptake, but to a lesser extent in the mutants. Finally, 30-minute bath application of 20 µM amphetamine showed that mutants had a smaller readily releasable vesicular pool of dopamine. In conclusion, the A313V mutation heightened evoked dopamine release, slowed uptake, and blunted the effect of DAT-blocking drugs. These data suggest that the A313V mutation reduces sensitivity to uptake inhibition by amphetamine and cocaine, which could alter locomotor responses to these drugs. Future studies will assess behavioral effects of DAT blockers and aim to develop treatments for mutations that cause DAT deficiency syndrome.

3-K-43 Can kappa opioid receptor antagonism normalize biochemical and behavioral phenotypes in mice expressing the ADHD-associated dopamine transporter variant Val559?

Felix Mayer[1], Paul Gresch[1], Adele Stewart[1], Maximilian Rabil[1], Roxanne Vaughan[2], Randy Blakely[1]
[1]Florida Atlantic University Brain Institute, [2]University of North Dakota

Extracellular dopamine (DA) is tightly regulated by the presynaptic dopamine (DA) transporter (DAT). The rare DAT Val559 variant has been identified in individuals with attention-deficit hyperactivity disorder (ADHD), bipolar disorder and autism. Behavioral alterations of Val559 mice include waiting impulsivity, altered locomotor responses to psychostimulants, and working memory deficits. The expression of Val559 can mediate a persistent, transporter-mediated outward leak of DA that can result in continuous stimulation of presynaptic D2-type autoreceptors (D2ARs), leading to elevated surface levels of Val559 in the dorsal striatum. As presynaptic kappa opioid receptors (KOR) have also been shown to regulate DAT surface expression and activity, we are examining the potential that KOR antagonism may ameliorate phenotypes associated with in vivo Val559 expression. Using biochemical approaches, we find that the KOR agonist salvinorin A elevates wild-type and Val559 DAT surface trafficking and enhances phosphorylation at threonine 53 (Thr53), a site that has been linked to DAT-mediated reverse transport and trafficking. Conversely, the KOR antagonist nor-binaltorphimine (norBNI) reduces both the surface trafficking and Thr53 phosphorylation of Val559, but has no effect on wild-type DAT. Using fiber photometry and microdialysis, we found that mice expressing Val559 display blunted vesicular DA release that can be normalized with systemic administration of norBNI. Finally, norBNI appears to reverse cognitive impairments of Val559 mice and normalize aberrant motor behavior. Together, these data suggest that pharmacological modulation of KOR activity may provide a novel treatment for disorders linked to aberrant DA signaling.

3-K-44 The impact of melatonin on evoked release of extracellular dopamine in the striatum of CBA/CaJ and C57BL/6J mice

Siham Boumhaouad[1], Nezha Bouhaddou[2], Stefano Cataldi[1], Eugene Mosharov[1], Avery McGuirt[1], Jihane Balla[2], Khalid Taghzouti[2], David Sulzer[1]
[1]Columbia University, [2]University Mohammed 5

It was recently demonstrated that extracellular dopamine (DA) levels fluctuate in a diurnal manner, likely due to circadian variation in the activity of the dopamine transporter (DAT). This circadian pattern could be controlled by nonphotic time cues including melatonin (MEL) and the melatonin receptors 1A (MT1) and 1B (MT2), which are reported to regulate DAT availability in striatal synaptosomes. We are comparing the interaction between MEL and evoked DA release by cyclic voltammetry in striatal slices from MEL expressing (CBA/CaJ) and MEL deficient (C57BL/6J) mice. The animals are analyzed at two times during a 12:12 h light/dark cycle, ZT10 and ZT22, respectively the nadir and peak of the MEL cycle. Trunk blood is collected to measure MEL by enzyme-linked immunoassay (ELISA) and an immunohistochemistry assay (IHC) to measure striatal expression of DAT, MT1 and MT2. Evoked DA release and reuptake are analyzed in coronal striatal slices (250 µm) containing nucleus accumbens (NAc) and caudate-putamen (CPu) in response to a single pulse electrical stimulus applied every 1 min for 5 min per region. The findings are expected to quantify MEL-mediated effects on striatal DA release and reuptake, paving the way for investigation into how MEL drives the circadian regulation of DAT function.

3-K-45 Dopamine 2 receptors in the extended amygdala gate action selection in threatening situations

Laia Castell[1], Valentine Le Gall[2], Laura Cutando-Ruiz[3], Chloé Petit[4], Emma Puighermanal[3], Ha-Rang Kim[2], Daniel Jercog[2], Pauline Tarot[4], Adrien Tassou[4], Anne-Gabrielle Harrus[4], Marcelo Rubinstein[5], Patricia Janak[1], Régis Nouvian[4], Cyril Rivat[1], Cyril Herry

[1]Johns Hopkins University, [2]Univ. Bordeaux, Neurocentre Magendie U1215, F-33077, [3]Neurosciences Institute, Autonomous University of Barcelona, [4]INM, Univ. Montpellier, Inserm, F-34000, [5]Instituto de Investigaciones en Ingeniería Genética y Biología Molecu

Organisms engage in different forms of behavior to maintain the homeostatic balance and reproduce. Some of these responses are evolutionary conserved and can be divided into approach and avoidance behaviors. Several factors affect the learning of appropriate behavioral responses towards a relevant stimulus such as the internal state of the animal or the balance between memory specificity and generalization. The central extended amygdala (EA) is a neuronal continuum critically involved in the control of action selection. By conveying salience and valence, dopamine (DA) facilitates the encoding of discriminative learning between stimuli representing safety or threat. We recently uncovered that DA gates overgeneralization of conditioned threat responses through concomitant activation of DA D2 type receptors (D2R) in both the central amygdala (CEA) and the bed nucleus of the stria terminalis. Likewise, the rescue of D2R in the CEA in a D2R-/- mice, restores normal feeding behavior in an impulsivity test. Despite these evidences, genetically-identified neural circuits of the EA in which D2R signaling control action selection, remain largely unknown. To address this issue, we have generated a D2R conditional knock-out mice allowing us to inactivate selectively the D2R in the EA. Our results revealed that EA-D2R signaling modulates the expression of passive responses to threat-conditioning stimuli. We also found that EA-D2R facilitates extinction of threat conditioned stimulus and is required for active avoidance learning. Furthermore, EA-D2R reshape feeding behavior specifically when the internal state of the animal is compromised. Our work suggests that, EA- D2R signaling in distinct neural circuits contribute to the optimization of the appropriate behavioral response.

3-K-46 Systemic isradipine effectively reduces mean firing rates in dopamine neurons in the lateral substantia nigra in awake and freely moving mice

Johannes Boehm[1], Josef Shin[1], Lora Kovacheva[1], Jochen Roeper[1]
[1]Goethe University Frankfurt a.M.

The Dihydropyridine (DHP)-sensitive L-Type calcium channels Cav1.3 and Cav1.2 serve various physiological functions e.g. acceleration of pacemaking in the heart. Furthermore, these channels are expressed in the dopamine (DA) system, more specifically in the DA neurons in the substantia nigra (SN). Also, clinical long-term use of the DHP isradipine (ISR) as antihypertensives has been shown to lower the risk of Parkinson disease. Previous work from our group demonstrated that Cav1.3 channels function as linear full-range amplifiers of firing rates (FR) selectively in lateral DA SN neurons projecting to the dorsolateral striatum. In addition, this FR amplifier function of Cav1.3 channel is dampened by clinically relevant concentration of ISR, both in vitro in acute brain slices and in vivo in anesthetized mice. To extend our study further to awake freely moving mice, we performed in vivo extracellular electrophysiological recordings after chronically implanting 7 bundles of stereotrodes into the lateral SN (lSN) (C57BL/6N (N=3)). We compared the FR of pharmacologically identified DA SN neurons (i.e. average 55% inhibition of mean FR after Quinpirole i.p.) during open-field exploration before and after 15 minutes post i.p. administration of ISR (3mg/kg). In comparison to our in vivo experiments in anaesthetized mice, where the baseline mean FR of juxtacellularly labelled and identified DA neurons in the lSN was reduced by ca. 27% after ISR, we observed similar results in pharmacologically identified DA neuron in the lSN (ca. 30% inhibition; n=25; N=3) of awake and freely moving mice. Our results confirm that Cav1.3 channels act as linear amplifiers of FR in lSN DA neurons and that this function can be targeted by DHPs in clinically relevant concentrations in awake animals.

3-K-47 A Population-Wide G-protein Coupled Receptor Atlas of Spiny Projection Neurons Identifies Novel Modulators of Striatal Activity

Mia Apuschkin[1], David Svane-Petersen[1], Charles Ducrot[2], Rasmus Rydbirk[3], Tomasz Brudek[3], Susana Aznar Kleijn[3], Louis-Eric Trudeau[2], Ulrik Gether[1], Mattias Rickhag[1]
[1]University of Copenhagen, [2]Université de Montréal, [3]Research Laboratory for Stereology and Neuroscience, Bispebjerg-Frederiskberg Hospital

In Parkinson's disease (PD), progressive loss of dopaminergic innervation to striatum causes an imbalance in activity of striatal projection neurons (SPNs); dSPNs become hypoactive while iSPNs become hyperactive resulting in motor deficits manifested as tremor, bradykinesia and rigidity. While the dopamine receptors, which belong to the superfamily of G protein-coupled receptors (GPCRs), have been the foremost studied biological target in PD, other GPCRs expressed in SPNs are much less characterized. We envision that yet poorly characterized striatal GPCRs, other than the dopamine D1-receptor and D2-receptor, constitute a tractable approach to restore a balanced dSPN/iSPN activity in PD. For this purpose, we have generated a cell population-wide GPCR expression atlas from SPNs by combining fluorescence-activated cell sorting using D1R-TdTomato and D2R-GFP reporter mouse lines followed by extensive quantitative PCR (qPCR) arrays. Custom-made qPCR array analysis revealed several novel GPCRs with preferential expression in either dSPNs or iSPNs. Candidate GPCRs was detected in mouse striatal sections by use of RNA scope. Human translatability was supported by detection of candidate GPCRs in human striatal PD samples using RNA sequencing and most targets showed high expression levels underlining the importance of expression of these GPCRs. Overall, we present a comprehensive depiction of the GPCR repertoire in SPNs and identify novel modulators of striatal signaling with a therapeutic potential in PD.

3-K-48 Synapse-associated protein 102 and post-synaptic density 95 differentially shape dopamine D1 receptor signaling

Bassam Albraidy[1], Bradley Mischuk[1], Chantal Binda[1], Kirk Mulatz[2], Geneviève Laroche[2], Patrick Giguère[2], Jean-Claude Béïque[2], Mario Tiberi[1]
[1]Ottawa Hospital Research Institute, [2]University of Ottawa

Post-synaptic D1 dopaminergic G protein-coupled receptors (D1Rs) localized in dendritic spine of neurons critically modulate movement, reward, cognition, and learning. The molecular underpinnings that shape the neuronal signaling and trafficking properties of D1R leading to the modulation of aforementioned brain functions remain unclear. To address this issue, we performed yeast two-hybrid screens using the intracellular loop 3 (IL3) of D1R and D5R and identify the synapse-associated protein 102 (SAP102) as a D1R-specific interacting partner. As previous studies showed that the SAP102 related post-synaptic density 95 (PSD-95) specifically interacts with the cytoplasmic tail (CT) of D1R, we tested the hypothesis that SAP102-IL3 and PSD-95-CT interactions differentially regulate D1R signaling and trafficking properties. Our co-immunoprecipitation and confocal microscopy studies using native brain preparations and transfected cells further validated that D1R interacts with PSD-95 and SAP102 in vitro and in vivo. Confocal imaging in organotypic hippocampal cultures suggest a co-localization of D1R with either PSD-95 or SAP102-labeled dendritic spines. Interestingly, GloSensor assays and ELISA show that PSD-95 and SAP102 differentially regulate D1R-mediated cAMP formation and DA-dependent D1R internalization. Additionally, Presto-Tango assays show that SAP102 and PSD-95 both facilitate β-arrestin2 recruitment to D1R. Overall, our results show an important role of SAP102 and PSD-95 in shaping differentially D1R signaling signatures. Future studies could exploit the pharmacological potential of D1R-SAP102 and D1R-PSD-95 complexes in the design of novel therapeutic approaches to mitigate abnormal D1R signaling function reported in Parkinson's disease and Alzheimer's disease.

L - Dopamine and neuroplasticity

3-L-49 Midbrain dopamine neurons trigger hippocampal long term potentiation and contextual learning

Fares Sayegh[1], Camille Lejards[1], Lionel Dahan[1]
[1]Université Paul Sabatier - Toulouse 3

The classical unsupervised model of hebbian Long Term Potentiation (LTP) fails to explain why some events are learnt while others are not. An alternative model postulates that a neuromodulator may act as a teaching signal triggering LTP. We used optogenetic tools to specifically manipulate the dopamine pathway originating from the midbrain and innervating the dorsal hippocampus. We show that dopamine midbrain projections can trigger LTP at Schaeffer Collaterals in vivo, in anaesthetized mice. In order to test for the role of this pathway in contextual learning, we used a variation of the contextual fear conditioning in which the context and its association to the electric shock take place on two consecutive days. We show that inhibiting the midbrain to hippocampus dopamine pathway impairs contextual learning while its stimulation promotes it. We thus discovered a new form of LTP triggered by dopamine which is compatible with computational models of hippocampo-dependent learning and we show that midbrain dopamine projections to the dorsal hippocampus are involved in contextual learning. We propose that, when something has to be learnt, midbrain dopamine provides a teaching signal to the hippocampus which induces hippocampal LTP responsible for learning and memory.

3-L-50 Dosage-dependent impact of acute serotonin enhancement on transcranial direct current stimulation effects

Lorena Melo[1], Mohsen Mosayebi-Samani[1], Elham Ghanavati[1], Michael Nitsche[1], Min-Fang Kuo[1]
[1]Leibniz Research Centre for Working Environment and Human Factors

The serotonergic system has an important impact on basic physiological and higher brain functions. Acute and chronic enhancement of serotonin levels via selective serotonin reuptake inhibitor (SSRI) administration impacts neuroplasticity in humans, as shown by its effects on cortical excitability alterations induced by non-invasive brain stimulation, including transcranial direct current stimulation (tDCS). Nevertheless, the interaction between serotonin activation and neuroplasticity is not fully understood, particularly when considering dose-dependent effects. Our goal was to explore dosage-dependent effects of acute serotonin enhancement on stimulation-induced plasticity in healthy individuals. Twelve healthy adults participated in 7 sessions conducted in a crossover, partially double-blinded, randomized, and sham-controlled study design. Anodal and cathodal tDCS was applied to the motor cortex under SSRI (20 mg/40 mg citalopram) or placebo medication. Motor cortex excitability was monitored by single-pulse transcranial magnetic stimulation (TMS). Under placebo medication, anodal tDCS enhanced, and cathodal tDCS reduced, excitability for about 60-120min after the intervention. Citalopram enhanced and prolonged the facilitation induced by anodal tDCS regardless of the dosage while turning cathodal tDCS-induced excitability diminution into facilitation. For the latter, prolonged effects were observed when 40 mg was administrated. Conclusions: Acute serotonin enhancement modulates tDCS after-effects and has largely similar modulatory effects on motor cortex neuroplasticity regardless of the specific dosage. A minor dosage-dependent effect was observed only for cathodal tDCS.

3-L-51 NMDA receptor-related mechanisms of dopaminergic modulation of tDCS-induced neuroplasticity

Elham Ghanavati[1], Mohammad Ali Salehinejad[1], Lorena De Melo[1], Michael Nitsche[1], Min-Fang Kuo[1]
[1]Leibniz Research Centre for working environment (ifado)

Dopamine is a key neuromodulator of neuroplasticity and an important neuronal substrate of learning, and memory formation, which critically involves glutamatergic N-methyl-D-aspartate (NMDA) receptors. Dopamine modulates NMDA receptor activity via dopamine D1 and D2 receptor subtypes. It is hypothesized that dopamine focuses on long-term potentiation (LTP)-like plasticity, ie reduces diffuse widespread but enhances locally restricted plasticity via a D2 receptor-dependent NMDA receptor activity reduction. Here, we explored NMDA receptor-dependent mechanisms underlying DAergic modulation of LTP-like plasticity induced by transcranial direct current stimulation (tDCS). Eleven healthy, right-handed volunteers received anodal tDCS (1 mA, 13 min) over the left motor cortex combined with DAergic agents (the D2 receptor agonist bromocriptine, levodopa (L-Dopa) for general dopamine enhancement, or placebo), and the partial NMDA receptor agonist D-cycloserine (CYC; dosages of 50, 100 and 200 mg, or placebo). Cortical excitability was monitored by transcranial magnetic stimulation-induced motor-evoked potentials. Low-dose CYC alone did not relevantly change anodal tDCS-generated LTP-like plasticity, while medium dosage prolonged and high dosage CYC diminished after-effects of stimulation. L-Dopa or bromocriptine alone reversed or abolished anodal tDCS-induced excitatory plasticity respectively, which was re-established by medium-dose CYC. Adding low dosage CYC did not alter the effects of bromocriptine and L-Dopa, while high-dose CYC abolished the after-effects.

These results suggest that diffuse LTP-like plasticity is counteracted upon via D2 receptor-dependent reduction of NMDA receptor activity.

M – Dopamine and behavior

3-M-52 Early-life exposure to antibiotics affects mesocorticolimbic circuitry and drug response in adult rats in a sex-dependent manner.

Camila González-Arancibia[1], Victoria Collio[1], Marcela Julio-Pieper[2], Ramón Sotomayor-Zárate[1], Javier Bravo[2], Francisco Silva-Olivares[1], Paula Montaña-Collao[1], Jonathan Martínez-Pinto[1]
[1]Universidad de Valparaíso, [2]Pontificia Universidad Católica de Valparaíso

Bacterial colonization of the infant gut begins at birth when partum exposes the newborn to a set of bacteria coming from the mother's gut. On the other hand, low microbial richness and diversity of gut microbiota are associated with several pathologies, including drug addiction. Preliminary results show that oral administration of non-absorbable antibiotic cocktail to a pregnant Sprague-Dawley dam from E18 until PD7, lowers gut microbial diversity and richness, and affects dopamine receptor expression at nucleus accumbens and ventral tegmental area in male offspring at P35. Thus, the aims of this study are to evaluate if early-life exposure to antibiotics (ELEA) impacts drug-seeking behavior, locomotor activity, in vivo striatal dopamine release, 3,4-dihydroxyphenylacetic acid (DOPAC) content, and dopamine receptors levels, in male and female adult offspring of dams treated with antibiotics in the peripartum. Control animals came from dams given only vehicle. ELEA increased preference for methylphenidate (MPH) in the adult male, while ELEA females showed a lower preference for MPH than their control. ELEA female rats had higher locomotor activity than ELEA males, release more striatal dopamine after MPH administration and showed lower ventral tegmental area's DOPAC levels than control females. Finally, protein analysis revealed expression changes of dopamine receptors 1 and 2, and tyrosine hydroxylase throughout the mesocorticolimbic circuit of ELEA rats, in comparison to their respective controls, Altogether, these results suggest that ELEA alters the development of the microbiota-gut-brain axis affecting the mesocorticolimbic structure, and hence, the response to drugs, in a sex-dependent manner in the adult rats.

3-M-53 Dopamine and norepinephrine signaling differentially mediate the exploration-exploitation tradeoff

Cathy Chen[1], Sijin Chen[1], Becket Ebitz[2], Nicola Grissom[1]
[1]University of Minnesota, [2]Université de Montréal

In an uncertain world, we balance two goals: exploiting rewarding options when they are available, and exploring potentially better alternatives. One neuromodulatory system that has been implicated in mediating the transition between exploration and exploitation is the catecholamine system, in particular, norepinephrine (NE) and dopamine (DA). Although both molecules have been implicated in decision making, their contributions have not been directly compared. When each neuromodulatory system is examined in isolation, they have been assigned similar roles in the latent cognitive processes that mediate exploration and exploitation. To understand the differences and overlaps of the role of these two catecholamine systems in regulating exploration and exploitation, a direct comparison using the same dynamic decision making task is needed. Here, we ran mice in a restless two-armed bandit task and systemically administered a NE beta-receptor antagonist (propranolol), NE beta-receptor agonist (isoproterenol), a nonselective DA receptor antagonist (flupenthixol), and a nonselective DA receptor agonist (apomorphine) and examined changes in exploration. We found that modulating NE

and DA receptor function had opposing effects on exploration - decreasing NE beta receptor activity or increasing DA receptor activity decreased exploration and resulted in stickier behaviors. Fitting a reinforcement learning model revealed that changes in exploration through manipulating NE and DA were due to changes in different latent processes. Together, these findings suggested that the mechanisms that govern the transition between exploration and exploitation are sensitive to changes in both catecholamine functions and revealed differential roles for NE and DA in regulating exploration.

3-M-54 Dopamine facilitates reward seeking in part by maintaining arousal

Saleem Nicola[1], Marcin Kazmierczak[1]
[1]ALBERT EINSTEIN COLLEGE OF MEDICINE

Dopamine facilitates approach to reward via its actions on dopamine receptors in the nucleus accumbens. For example, blocking either D1 or D2 dopamine receptors in the accumbens reduces the proportion of reward-predictive cues to which rats respond with cued approach. Recent evidence indicates that accumbens dopamine also promotes wakefulness and arousal, but the relationship between dopamine's roles in arousal and reward seeking remains unexplored. Here, we show that the ability of systemic or intra-accumbens injections of the D1 antagonist SCH23390 to reduce cued approach to reward depends on the animal's state of arousal. Handling the animal, a manipulation known to increase arousal, was sufficient to reverse the behavioral effects of the antagonist. In addition, SCH23390 reduced spontaneous locomotion and increased time spent in sleep postures, both consistent with reduced arousal, but also increased time spent immobile in postures inconsistent with sleep. In contrast, the ability of the D2 antagonist haloperidol to reduce cued approach was not reversible by handling. Haloperidol reduced spontaneous locomotion but did not increase sleep postures, instead increasing immobility in non-sleep postures. Our results suggest that deficits in operant performance caused by impaired dopamine function can be jointly explained by reduced arousal and motor function rather than disrupted motivation or reward.

3-M-55 Souris-City: a multi-environment for understanding the social basis of inter-individual variability and drug vulnerability in mice.

Sophie Fayad[1], Nicolas Torquet[2], Lauren Reynolds[1], Fabio Marti[3], Stefania Tolu[4], Claire Nguyen[1], Sarah Mondoloni[5], Robin Justo[1], Steve Didienne[1], Nicolas Debray[3], Louis Brasselet[1], Nicolas Renier[6], Alexandre Mourot[1], Philippe Faure[1]
[1]ESPCI - PSL, [2]Institute of genetic and molecular and cellular biology, [3]Sorbonne Université, [4]University of Paris, [5]University of Lausanne, [6]Brain and Spinal Cord Institute

Behavioral differences among individuals are ubiquitous in animal studies. When consistent across time and contexts, they define animal personality. In adulthood, environmental factors, such as social context, play a major role in personality adaptation. However, the underlying neurophysiological mechanisms are poorly understood and bring promising leads towards understanding inter-individual differences in drug vulnerability. We aim at unraveling the social determinants of inter-individual variability and drug vulnerability, using a semi-natural environment, "Souris City", where mice are living in large social groups and individually tracked for several weeks. This system combines a social homecage and a T-maze with individual access. It allows us to observe correlations between decision-making strategies in the T-maze and behavioral traits in the social zone, to describe individual profiles. Using juxtacellular recordings in anaesthetized mice, we found that individuals with distinct behavioral traits also display differences in the spontaneous activity of their Ventral Tegmental Area (VTA)

dopaminergic (DA) neurons. We next investigated whether these mice also showed differences in their response to nicotine exposure, by recording VTA DA neurons responses to intravenous nicotine injections. Using c-fos immunoreactivity in cleared whole brains, we further sought for inter-individual differences in global responses of brain structures to intraperitoneal nicotine injections. Finally, we looked into the impact of nicotine intake on individual behavior, either through voluntary consumption, or by chronic administration via subcutaneous osmotic pumps. This combinatorial approach allows to address key questions on individual personality and vulnerability to nicotine.

3-M-56 Mesolimbic dopamine and interpeduncular-tegmentum circuitry dynamics of social novelty processing and adaptive learning

Susanna Molas[1], Timothy Freels[1], Rubing Zhao-Shea[1], Melanie Barbini[1], Andrew Tapper[1]
[1]University of Massachusetts Chan Medical School

Novelty and adaptive learning with familiarity represent conserved motivational drives critical for adaptation to changes in the environment and goal-directed behaviors. Accumulating data indicates midbrain dopamine (DA) systems guide approach/avoidance behaviors to different types of novelty. In addition, we previously demonstrated that the interpeduncular nucleus (IPN) represents a neuroanatomical substrate for familiarity signaling and novelty preference. The IPN is a component of the habenulo-interpeduncular pathway connecting limbic forebrain with midbrain/hindbrain areas. However, the precise real-time activity patterns of DA systems and IPN circuits causally linked to novelty and familiarity responses is unclear. Using fiber photometry recordings with biosensors in mice we have found that both ventral tegmental area (VTA) DAergic neuron activity and ventral striatum DA release are significantly engaged by social novelty and respond following classical reward prediction error signals as novel social stimuli become familiar. In contrast, IPN GABAergic activity is inhibited during novel social investigations but engaged by familiarity. Circuit-tracing analysis reveals that IPN GABAergic neurons strongly innervate the laterodorsal tegmentum (LDTg), which sends excitatory inputs to VTA. This IPN-LDTg circuit conveys inhibitory dynamic patterns during novel stimuli interactions. Moreover, time-locked optogenetic stimulation of the IPN GABAergic-LDTg pathway bidirectionally modulates novelty seeking behavior, indicating the IPN, via the LDTg, influences DA transmission to control novelty responses. These data identify new circuitry within the novelty network that may be key in understanding disrupted social novelty responses observed in neurological disorders.

3-M-57 Topographic organization of hippocampal functional connectivity is linked to the dopamine D1 receptor across the adult lifespan

Kristin Nordin[1], Jarkko Johansson[1], Filip Grill[1], Farshard Falahati[1], Robin Pedersen[1], Micael Andersson[1], Anna Rieckmann[1], Alireza Salami[1]
[1]Umeå University

Cognitive decline in aging has numerous biological sources, including changes in hippocampal (HC) function. Past studies have demonstrated topographic principles of functional connectivity (FC) along multiple HC axes in young adults. Yet, age-related alterations in these remain unexplored. Given that dopamine (DA) is key to memory formation, and exerts an enhancing effect on signal-to-noise ratio of neural signaling, it is possible that age-related differences in the DA system might be related to alterations in topographic HC FC. Here, we used connectopic mapping to identify gradients of cortical FC within the HC across the adult lifespan, using the DyNAMiC dataset including resting-state fMRI and [11C]SCH23390 PET data for estimates of DA D1 receptor availability (D1DR) in 180 individuals (20-79

years). A principal anterior-to-posterior gradient was identified, along with a second-order long-axis gradient spreading from the middle HC towards anterior and posterior ends. A third-order gradient tracked the HC medial-lateral axis, anatomically constituted of its cytoarchitectural subfields. Strongest effects of age were evident in the primary long-axis gradient, which predicted episodic memory (EM) in young adults, and was modulated by D1DR across the sample. Compared to older adults with low EM, older adults with high EM displayed a gradient more similar to that of young and middle-aged adults. To some extent, D1DR predicted topographic properties of all three gradients within older adults, suggesting a primary dependence on D1DR modulation in a context of altered functional HC integrity. Our results provide evidence for age-related alterations along multiple HC axes and highlight DA as a promising modifying factor in maintaining topographic HC organization in older age.

N –Other

3-N-58 L-type channel control of DA release is gated by endogenous regulators, can we utilise them as neuroprotective strategies against Parkinson's disease?

Katherine R Brimblecombe[1], Stephanie J Cragg[1]
[1]*University of Oxford*

SNc and VTA dopamine (DA) neurons, which project to dorsal and ventral striatum respectively differ in a number of ways. Notably in their high and low sensitivity to parkinsonian degeneration respectively. We have previously identified that DA release is differentially gated by L-type voltage-gated Ca2+ channels (LTCC) in the dorsal and ventral striatum. Given that LTCC function has been identified as a stressor of DA neurons at risk for parkinsonian degeneration we are interested in identifying the molecular mechanisms regulating LTCC function. Using fast-scan cyclic voltammetry in acute ex-vivomouse brain to access mechanisms regulating LTCC control of DA release across striatal territories in both sexes. We identify calbindin-D28K as limiting LTCC function in a regionally and sexually divergent manner: D2-receptors and DA-transporters as negative and positive regulators of LTCC respectively and lastly find that targeting α2δ subunits with gabapentinoid drugs limits LTCC function without compromising DA release. Therefore LTCC-function can be dynamically and locally regulated which may prove critical for future neuroprotective strategies.

3-N-59 Dopaminergic reward and performance prediction error signal are gated during courtship

Andrea C Roeser[1], Vikram Gadagkar[1], Pavel A Puzerey[1], Brian Kardon[1], Anindita Das[1], Jesse H Goldberg[1]
[1]*Columbia University*

How do social interactions affect dopaminergic (DA) responses to rewards and performance outcomes? We used electrophysiology and fiber photometry to record DA signals in two mesostriatal pathways as thirsty male songbirds sang alone and to females. When alone, singing-related performance error signals were restricted to a song-specialized mesostriatal pathway; reward prediction error signals were observed globally. When singing to a female, DA responses to both water reward and song performance outcomes were diminished and were instead driven specifically by female calls that interrupted the song. Together, we discover that reward and performance error signals are differentially routed through distinct DA pathways, that DA signals dynamically change their tuning during courtship, and that an affiliative social interaction, when precisely timed, activates distinct DA systems.

Poster Session 4

A – Dopamine, motivation, reward and addiction

4-A-1 Nucleus accumbens dopamine levels appear to control the ability of sexually satiated male rats to respond to a sexual stimulus

Ana Canseco-Alba[1], Gabriela Rodríguez-Manzo[1]
[1]Cinvestav

Dopamine (DA) release in the nucleus accumbens (NAcc) has been suggested to be involved in sexual motivation and behavior. In sexually experienced male rats, the mere exposure to an inaccessible sexually receptive female and copulation itself, increases DA levels in the NAcc. Sexual satiety consists of a long-lasting sexual behavior inhibition (up to 72 hours) that appears in response to repeated ejaculation in the course of sustained ad libitum copulation. 24 hours after its establishment, the majority of sexually exhausted rats (2/3) do not show sexual activity in the presence of a receptive female. It has been shown that sexually satiated rats have a decreased sexual motivation. The aim of this work was to determine NAcc DA concentrations during sexual satiety development and 24 hours later, during the well established sexual inhibitory state. NAcc DA and its metabolites, DOPAC and HVA, were monitored by in vivo microdialysis in freely moving male rats. These levels remained increased during copulation and began to decline once copulation ended. Basal DA and its metabolites' levels were significantly reduced 24 hours after copulation to satiety, as compared to the initial basal levels. At this moment, presenting a receptive female behind a barrier did not induce the typical NAcc DA elevation but there was a decrease that persisted when the satiated male had access to the female, with which it did not copulate. Systemic injection of an anandamide dose reversing satiety, induced an increase in NAcc DA slightly above the basal levels, which coincided with sexual behavior display by the sexually satiated rats. Results suggest that NAcc DA levels have to reach a threshold for the male to be able to respond to the sexual stimulus that represents the receptive female.

4-A-2 Effects of endogenous orexin and dynorphin corelease on ventral tegmental dopamine neuronal activity

Aida Mohammadkhani[1], Min Qiao[1], Stephanie Borgland[1]
[1]University of Calgary

Dopamine neurons in the ventral tegmental area (VTA) respond to motivationally relevant cues and are key targets of addictive drugs. Orexins (ox; also known as hypocretin) and dynorphin (dyn) are co-expressed lateral hypothalamic (LH) neuropeptides that project to VTA. While LHox promotes drug-seeking behavior, dynorphin inhibits drug-seeking behavior. Furthermore, these peptides have opposing effects on the firing activity of VTA dopamine neurons. Previous work in our lab implicated that exogenously application of ox and dyn, modulate different VTA dopaminergic projections. However, it is unknown if dynorphin inhibition of these circuits in opposition to LHox is driven by the LHox/dyn input, rather than other sources. This study sought to determine the effects of endogenous LHox/dyn release on VTA dopamine neuronal activity. We expressed channel rhodopsin2 selectively in LHox/dyn neurons and photostimulated terminals in the VTA while recording VTA neuronal firing using patch clamp electrophysiology. VTA dopamine neurons were labeled with biocytin during recordings and posthoc imaged for tyrosine hydroxylase expression. We showed a diverse response of LHox/dyn photostimulation on dopamine neuronal firing rate. A 30-Hz stimulation, increased firing in approximately 33% of neurons and decreased firing in approximately 55% of lateral VTA dopamine

neurons, an effect persisted in the presence of synaptic transmission blockers. SB334687, an ox1 receptor inhibitor or NorBNI, a kappa receptor inhibitor reversed the potentiation or inhibition of firing, respectively. Our findings provide evidence that LHox/dyn corelease may tune the output of the VTA by simultaneously inhibiting and activating different VTA projection neurons. Keywords: orexin, dynorphin, addiction, ventral te

4-A-3 Stereotyped behavior in rewarding scenarios in a mouse model of 16p11.2 hemideletion

Erin Giglio[1], Gerardo Rojas[1], Arielle Duerr[2], Jenelle Collier[1], Aaron Bastin[1], Max Ritchie[1], Mackenzie Lund[1], Ann Hajostek[1], Nicola Grissom[1]
[1]University of Minnesota, [2]University of St. Thomas

Neurodevelopmental disorders like autism spectrum disorder (ASD) have strong male biases in diagnosis and severity, but how sex differences interact with genetic contributors to ASD and lead to differential cognition is unclear. A mouse model of 16p11.2 hemideletion (del/+) - a copy number variation linked to neurodevelopmental diagnoses including ASD - has been shown to display male-specific vulnerabilities in motivated behaviors. A key feature of ASD is the development and expression of stereotypic behaviors, which employ the same neural systems involved in social and non-social reward learning. 16p11.2 del/+ mice have previously been demonstrated to have changes in their striatal molecular function and learning deficits. While 16p11.2 del/+ mice have been examined on a systems level, they have yet to be examined on a neurotransmitter level. In order to probe how catecholamines affect 16p11.2 del/+ mice, we plan to employ an amphetamine-induced locomotor assay where female and male mice will be treated with increasing doses of amphetamine. We are interested in the time course of behavioral sensitization and the development of stereotypic behaviors with each increasing dose. Our previous observations have shown male del/+ mice rotate in response to increasing doses of amphetamine whereas male wildtypes sensitize through increased locomotion. These results suggest there are significant reductions and/or imbalances in dopamine function in the striatum which could drive the expression of stereotypic/repetitive behaviors and bias reward guided behaviors. These results have important implications for the role of dopamine in regulating reward guided learning and decision-making in a mouse model of 16p11.2 del+/.

4-A-4 Hormonal regulation of dopaminergic signaling and value-based decision-making

Carla Golden[1], Andrew Mah[1], Christine Constantinople[1]
[1]New York University

Despite the broad influence of gonadal hormones throughout the brain, little is known about how they influence dopamine activity during value-based decision-making. In reinforcement learning models, a subject learns the value of actions from experience and updates those values based on reward prediction errors (RPE)-the difference between received and expected rewards. Dopamine released in the nucleus accumbens core (NAcc) represents RPEs for learning and is implicated in response vigor. We sought to determine whether gonadal hormones modulate learning by modulatingdopaminergic RPEs in the NAcc. We studied female rats over their estrous cycle as they performed a novel self-paced temporal wagering task wherein they deliberate between waiting for an unpredictable reward or starting a new trial. The task provides a readout of the rats' subjective value of the reward (how long they are willing to wait) as well as their motivation (time to initiate trials). We manipulated reward expectations by varying the reward magnitudes in blocks of trials (local reward context). During

proestrus and estrus, when estradiol and progesterone are high, subjective value and motivation are more sensitive to local reward context. We identified three novel molecular targets of hormonal regulation involved in dopamine reuptake in the NAcc (Smpd3, Sl6a3, and Sl6a4) that are downregulated in proestrus and estrus, suggesting that gonadal hormones might enhance extracellular dopamine levels. Dopaminergic encoding of RPEs measured with fiber photometry of GRABDA is indeed enhanced. Altogether, this suggests that gonadal hormones promote the downregulation of proteins that mediate dopamine reuptake in the NAcc in proestrus and estrus, leading to enhanced RPEs and learning about reward context.

4-A-5 Dopamine-induced changes in reward and punishment learning characterize Impulse Control Disorder

Brittany Liebenow[1], Paul Sands[1], Angela Jiang[1], Emily DiMarco[1], Mary Moya-Mendez[2], Adrian Laxton[1], Stephen Tatter[1], Mustafa Siddiqui[1], Ihtsham Haq[3], Kenneth Kishida[1]
[1]Wake Forest School of Medicine, [2]Duke University School of Medicine, [3]University of Miami Miller School of Medicine

Impulse control disorder (ICD) is a sub-class of behavioral addictions caused by dopaminergic therapies used to treat Parkinson's disease (PD). ICD patients exhibit a dopaminergic therapy induced increase in risky decision-making behavior that persists even in the face of repeated adverse outcomes. How rewards and punishments are processed in these patients is not well understood. We recruited PD patients with a history of ICD ('ICD', N=18) and without ('non-ICD', N=11). Participants completed a Probabilistic Reward and Punishment task while on or off their dopaminergic medications (on-DM or off-DM, respectively). In this task, participants repeatedly made choices leading to uncertain monetary gains and losses. We fit a Valence Partitioned Reinforcement Learning model to each participant's behavior to measure how ICD and non-ICD groups learned from appetitive and aversive feedback. The ICD group learned faster from surprising rewards when on-DM compared to off-DM ($p<0.001$); this DM-induced change is not observed in the non-ICD group. In the off-DM state, there was no difference in learning rates to punishing feedback between the ICD and non-ICD groups. However, on-DM was associated with an increase in the punishment learning rates in both groups ($p<0.001$) that was significantly lower in the ICD group compared to the non-ICD group ($p<0.01$). Further, DM increased the influence of expectations about future rewards ($p<0.05$) and punishments ($p<0.001$) in the non-ICD group, a change that was not observed in patients with ICD. Our results suggest that patients with ICD are more sensitive to dopaminergic representations of immediate rewards, less sensitive to the effects of immediate punishments, and are blunted in their consideration of future negative consequences.

4-A-6 Dopamine-glutamate receptor heteromerization as a common molecular substrate for substance use disorder and comorbid depression

Marie-Charlotte Allichon[1], Vanesa Ortiz[2], Paula Pousinha[2], Sebastian Fernandez[2], Andry Andrianarivelo[1], Anna Petitbon[3], Alexis Bemelmans[4], Ying Zhu[5], Jozsef Meszaros[5], Jonathan Javitch[5], Pierre Trifilieff[3], Jacques Barik[2], Peter Vanhoutte[1]
[1]Sorbonne Université, [2]Institut de Pharmacologie Moléculaire et Cellulaire, [3]University of Bordeaux, [4]Atomic Energy and Alternative Energies Commission, [5]Columbia University

Drug addiction is defined as a compulsive pattern of drug-seeking/taking behaviors, with recurrent episodes of abstinence and relapse. Addictive drugs increase dopamine in the nucleus accumbens (NAc), where it persistently shapes excitatory glutamate transmission, thereby hijacking natural reward

processing. We provide evidence, from mice to humans, that an underlying mechanism relies on a drug-evoked heteromerization of glutamate NMDA receptors with dopamine receptor 1 (D1R) or 2 (D2R) triggered by both psychostimulants and opiates. Using temporally-controlled inhibition of D1R-NMDAR heteromerization, we show their selective implication in early phases of cocaine-evoked, whereas preventing D2R-NMDAR heteromers blocked the persistence of these adaptations. Interfering with these heteromerizations spared natural reward processing. Because the high prevalence of comorbidities between addiction and mood disorders suggests that brain dysfunctions underlying drug addiction and other psychiatric disorders may rely on partly shared mechanisms, we asked whether receptor heteromers in the NAc could constitute a common molecular switch in addiction and depression. Using the chronic defeat stress paradigm as a preclinical model of depression, we found that mice developing depressive-like behavior, but not resilient mice, exhibit an increased dopamine-glutamate receptor heteromerization, which blockades fully prevents the onset and persistence of depressive-like symptoms. These findings contribute to a better understanding of common molecular mechanisms underlying addiction and depression and uncover dopamine-glutamate heteromer as targets with potential therapeutic value for multiple psychiatric diseases associated with alterations in dopamine and glutamate-dependent transmissions

4-A-7 Respective roles of the distinct populations of medium spiny neurons of the nucleus accumbens in reward processing and feeding behavior.

Petitbon Anna[1], Fois Giulia[2], Ducrocq Fabien[3], Contini Andrea[1], Ortole Rodrigue[1], De Smedt-Peyrusse Véronique[4], de Kerchove d'Exaerde Alban[5], Giros Bruno[6], Chaouloff Francis[7], Ferreira Guillaume[1], Trifilieff Pierre[1]

[1]NutriNeuro UMR 1286 INRAE, [2]Institut des Maladies Neurodégénératives, UMR 5293, [3]UMC Utrecht, [4]NutriNeuro UMR 1286, [5]ULB Neuroscience Institute, Université Libre de Bruxelles, [6]Douglas Hospital, McGill University, [7]NeuroCentre Magendie INSERM U1215

Compulsive eating, found in some forms of obesity, and anorexia nervosa (AN) can be considered as opposite pathologies in light of their symptomatology, i.e. feeding behaviors and physical activity . Even though little is known regarding the pathophysiological mechanisms of these diseases, accumulating evidence points to a dysfunction of the mesolimbic pathway. Our study aimed at addressing whether manipulating the activity of neuronal subpopulations of the Nucleus Accumbens (NAc), namely D1 or D2 expressing medium spiny neurons (D1-MSNs ; D2-MSNs), could be sufficient to mimic some symptomatic dimensions of compulsive eating and AN. Using a chemogenetic approach, we found that acute activation of eith D1 or D2-MSNs leads to opposite findings, supporting a functional dichotomy. While chronic activation of D1-MSNs leads to excessive wheel running, animals counteract this energetic expenditure by increasing their food consumption. On the contrary, chronic activation of D2-MSNs triggers weigh gain due to low physical activity and increased food consumption. Thanks to a unique double-transgenic mouse model, we were able to concomitantly manipulate both subpopulations of the NAc. We show that simultaneous activation/inhibition of D1-MSNs/D2-MSNs overrides the compensatory mechanism observed under D1-MSNs activation alone, resulting in weight loss due to excessive physical activity regarding food intake. In opposite, chronic inhibition/activation of D1- and D2-MSNs induces weight gain due to excessive energy intake in regard to activity-mediated energy expenditure. Our results suggest that the balance between D1- and D2-MSNs activity bidirectionally modulates energetic homeostasis and that an alteration of such balance could promote the occurrence of eating disorders.

4-A-8 Expression of the glutamate transporter GLT-1 in dopaminergic axons in the medial shell and age-dependent consequences of its deletion on behavioral sensitization to amphetamine

Kathryn Fischer[1], Ang Sherpa[2], Chiye Aoki[2], Sophie Griswold[3], Paul Rosenberg[4]
[1]Indiana University School of Medicine, [2]New York University, [3]Brandeis University, [4]Harvard Medical School and Boston Children's Hospital

A subpopulation of dopamine (DA) neurons use glutamate as a neurotransmitter, and a primary target of these neurons is the medial shell of the accumbens. We investigated whether dopaminergic axons in this region express the glutamate transporter GLT-1, and the effect of conditionally knocking out GLT-1 in DA neurons in response to amphetamine (AMP) at different ages up to 22 months. Double label EM immunocytochemistry (EM-ICC) for tyrosine hydroxylase (TH) and GLT-1 were used to detect expression of GLT-1 in dopaminergic axons in the medial shell. The GLT-1 gene was inactivated in dopaminergic neurons using a conditional GLT-1 knockout (GLT-1flox/flox) and DAT-IRES-Cre. Locomotor sensitization to AMP (3 mg/kg) was assessed in 8-22 month old males using a five day induction protocol and a challenge 10-14 days after induction. EM immunocytochemistry revealed double labeling of axons for TH and GLT-1. Knockout of GLT-1 restricted to DA neurons using DAT-IRES-Cre (datGLT-1 KO) eliminated the anti-GLT-1 labeling of TH positive axons in the medial shell ($p<0.0001$). DatGLT-1 KO mice showed a significantly blunted response to AMP on day 1 induction testing compared to WT mice at 8-9 months. At 11+ months, WT mice show a significantly blunted locomotor response to AMP on day 1 induction testing compared to datGLT-1 KO mice. This reversal of effect appeared to be due to WT mice showing a reduction in their response to AMP as they aged, whereas responses to AMP displayed by datGLT-1 KO mice remained constant. These data demonstrate that GLT-1 is expressed in DA neurons, and suggest that GLT-1 deletion restricted to DA neurons alters the effect of aging on sensitivity to AMP.

4-A-9 Distinct dopamine signals for habit vs. goal-directed behavior in the ventral tegmental area

Robin Magnard[1], Patricia Janak[2], Youna Vandaele[1]
[1]Johns Hopkins University; Janak Lab, [2]Johns Hopkins University ; Johns Hopkins School of Medicine

Habitual performance is commonly considered in light of dorsolateral striatum and its nigral dopaminergic (DA) input. However, how ventral tegmental area DA neuron activity might also differentially signal habitual versus goal-directed performance remains largely unknown. To address this question, we tested rats in two operant procedures, a lever insertion fixed-ratio 5 (LI5) task and a lever retraction fixed-ratio 5 (LR5) task. Here, the timing of lever insertion (LI) and retraction (LR) is varied so that one is a relevant cue signaling reward (sucrose 20%) availability while the other cue is made irrelevant. We then used satiety-induced devaluation to test whether behavior was goal-directed or habitual. Finally, we compared activity of DA neurons via in vivo fiber photometry recording after VTA infusion of Cre- dependent GCaMP6f virus in adult TH-Cre rats performing either the LR5 or LI5 task. We found rats quickly developed habitual behavior and chunking in the LR5 task in which the LR cue predicts immediate reward delivery. We further observed rapid shifts in activation of DA VTA neurons from reward retrieval to the earlier LR cue, followed by decreases in cue-related DA signals across repeated trials as performance become automatic and habitual. In contrast, behavior in the LI5 task was goal-directed, and cue-induced DA activation remained relatively constant across trials and sessions, when reward availability was signaled by the LI cue, more distal from reward delivery. These results show manifest task differences in DA signaling that appear consistent with differences in habitual versus goal-directed control of behavior.

4-A-11 Role of D2 receptor-positive ventral tegmental area dopamine neurons in effort-related motivation for food-seeking

Yoshio Iguchi[1], Shigeki Kato[1], Kazuto Kobayashi[1]
[1]Fukushima Medical University

Ventral tegmental area (VTA) dopamine neurons are critically involved in a variety of learning and motivation processes, which is reflected at least in part the phenotypic diversity of VTA neurons of which projection targets and expression molecules are distinct. We investigated how the activity of D2 receptor-positive dopamine neurons in VTA contributes to the control of the effort-related motivation of instrumental behavior reinforced by natural reward (food pellets). We developed a Drd2-Cre rat line (Nonomura et al., Neuron, 2018), and then induced the expression of the chloride ion channel derived from Caenorhabditis elegans (GluCLα/β; Lerchner et al., Neuron, 2007) in the VTA using two type of adeno-associated virus 2 (AAV2) vectors containing a double-floxed, inverted open reading frame sequence for GluCLα or GluCLβ. We reversibly inhibited the activity of the target neurons in a ligand (ivermectin) -dependent manner and examined the effects on motivation-related parameters underlying instrumental behavior estimated based on an economic demand-supply model (e.g., Mahler et al., J Neurosci., 2019). Ivermectin treatment, microinjection into the VTA as well as systemic administration, increased demand elasticity to the reward prices (number of lever presses required to obtain a unit of reward) that we changed systematically within a training session. Ivermectin systemic administration also increased reward consumption under low-effort conditions. This result highlights the multifaceted role of the D2 receptor-positive VTA neurons in effort-related motivation for food-seeking.

4-A-12 Characterizing value signals in the human midbrain

Jae-Chang Kim[1], Lydia Hellrung[1]
[1]University of Zurich

Although a substantial body of evidence has associated the dopaminergic midbrain with processing subjective value and prediction errors, the exact nature of these value signals remained unclear. Using neuroimaging and liquid reward and punishment, here we dissociate two proposals from the animal literature. The more generally accepted single dimension hypothesis posits that the dopaminergic midbrain encodes subjective value across the full spectrum of positive and negative outcomes. By contrast, the dual dimension hypothesis proposes that value coding by the dopaminergic midbrain is limited to evidence about positive outcomes. Here, 41 participants performed a Pavlovian conditioning task in which different cues were associated with different probabilities (p=0.0, 0.5 or 1.0) of appetitive, neutral, or aversive liquid. We find value prediction error signals for reward but not for punishment at the time of the outcomes. At the time of cues, phasic responses increased with probability for cues associated with reward but not for cues associated with punishment. While these activations partly overlapped with the outcome-induced activations, they tended to locate more ventrally. Moreover, dorsomedial regions showed decreasing responses with probability specifically for reward-associated cues, reflecting an inverse value signal, which constitutes a key ingredient for computing prediction errors. Together, our data indicate value coding primarily for the appetitive domain, in line with the dual dimension hypothesis.

4-A-13 Synaptogyrin-3 Modulates Dopamine Release and Selectively Reduces Cocaine Self-Administration

Emily Peck[1], Paige Estave[1], Katherine Holleran[1], Brianna George[1], Sara Jones[1]
[1]Wake Forest University

Synaptogyrin-3 (SYG3) is a synaptic vesicle protein highly expressed in dopamine-containing neurons that directly interacts with the dopamine transporter (DAT), suggesting a role in synaptic dopamine dynamics. The DAT is the primary reinforcing site of cocaine, and chronic cocaine exposure alters DAT function, expression, and dopamine release dynamics. We tested the hypothesis that chronic cocaine exposure disrupts SYG3 function, resulting in DAT alterations that drive excessive cocaine taking. Rats were trained to self-administer cocaine and tested on a progressive ratio (PR) schedule of reinforcement. Western blots showed a significant positive correlation between SYG3 and DAT protein levels and a significant negative correlation between SYG3 and PR breakpoint in the ventral tegmental area and nucleus accumbens. Thus, we virally-overexpressed SYG3 in VTA dopamine neurons of cocaine-naive rats to assess the effects on cocaine and sucrose self-administration, anxiety-like behavior, and learning assays. Additionally, nucleus accumbens dopamine terminal function was measured using ex vivo fast-scan cyclic voltammetry. SYG3 overexpression resulted in reduced cocaine responding on an extended access schedule and a lower PR breakpoint. SYG3 overexpression also reduced anxiety-like without altering sucrose self-administration, sucrose preference, or pairwise discrimination. While SYG3 overexpression did not affect dopamine uptake rate in cocaine-naive rats, it bimodally altered dopamine release--blunting release in response to high stimulation intensities but increasing release at low stimulation intensities. Together, these data provide evidence for SYGS's role as a regulator of dopamine kinetics and a potential target for pharmacotherapeutics to treat cocaine use disorder.

4-A-14 Dopamine and addiction theory

Roy Wise[1]
[1]National Institute on Drug Abuse

Early theories of addiction, food reward, and brain stimulation reward identified a role for dopamine in reinforcement; blockade of dopamine function blocked the reinforcing functions of amphetamine, cocaine, opiates, food, and brain stimulation reward. Against this theory were findings that dopamine antagonists blocked lever-pressing for food but did not block free-feeding; this suggested that dopamine was involved in motivation rather than reward. Other studies showed that decerebrate rats show signs of liking food; this (and a good deal of additional evidence) suggested that the dopamine system is not responsible for subjective pleasure. Contemporary thinking is that dopamine is important for two functions--reinforcement and motivation--but is not important for affective response to reinforcers. Reinforcement depends on glutamate- (and perhaps acetylcholine-) induced burst-firing of the dopamine system, along with its consequence, NMDA receptor-dependent long-term potentiation and depression in the striatum, whereas motivation depends on GABA- and hormone-modulated single-spike dopaminergic firing. A third factor--dopamine receptor down-regulation and associated loss of interest in non-habitual reward-predictors--also develops with a variety of addictions. These three factors--reinforcement, motivation, loss of interest--offer a definition of addiction that appears to generalize beyond addictive drugs to high-energy foods, social interactions, and gambling. Withdrawal symptoms--symptoms associated with abstinence from habitual rewards--are unique to various drug classes and other rewards. Each, with its physiological specifics, can define one but not all of the various addictions.

4-C-15 Inflammation and sensitization in Schizophrenia: A [11C]-(+)-PHNO PET Study

Carina Bum[1], Ana Weidenauer[1], Matthäus Willeit[1], Ulrich Sauerzopf[1], Martin Bauer[1], Lucie Bartova[1], Alina Kastner[1], Nics Lukas[1], Philippe Cecile[1], Neydherr Berroterán-Infante[1], Veronika Pichler[1], Pablo Rusjan[1], Markus Mitterhauser[1], Marcus Hacker[1], Wolfgan

[1]Medical university of vienna

Sensitization is a process in which repeated intermittent exposure to a stimulus results in an amplified reaction during consecutive exposures. We previously showed that schizophrenia is characterized by an endogenous sensitization of the dopamine system. It is assumed that inflammatory mechanisms contribute to the development of schizophrenia. Therefore, we performed an exploratory post hoc study to investigate the role of peripheral immune cells in healthy volunteers and patients with schizophrenia. We studied 28 healthy volunteers (14 female), who underwent a baseline and a post-amphetamine [11C]-( )-PHNO scan before and after a mildly sensitizing regimen of repeated oral amphetamine to measure D-amphetamine dopamine release. Twenty-one treatment naive patients with first-episode psychosis (6 female) underwent one baseline and one post-amphetamine scan. Leucocyte and fibrinogen were assessed in peripheral blood. In patients with schizophrenia peripheral fibrinogen predicted feeling alert, focused, energetic and outgoing upon D-amphetamine ($p<0.05$). No significant correlation between leukocytes and fibrinogen and dopamine release in any brain region in none of the groups was detected. However, leukocyte levels negatively predicted sensitization to d-amphetamine in the putamen ($r = -0.44$, $p = 0.03$) and on a trend-level in the SNVTA ($r = -0.42$, $p = 0.06$). Our findings suggest that inflammatory processes contribute to the development of D-amphetamine sensitization in healthy subjects and mediate the behavioral response to D-amphetamine in patients with schizophrenia. In conclusion, multimodal imaging studies focusing on dopamine release and immunological processes might shed further light on the pathogenic mechanisms of dopamine-related neuropsychiatric disorders.

4-C-16 The novel atypical antipsychotic cariprazine demonstrates dopamine D2 receptor-dependent partial agonist actions on rat mesencephalic dopamine neuronal activity

Sarah Delcourte[1], Charles Ashby Jr[2], Renaud Rovera[1], Béla Kiss[3], Nika Adam[4], Bence Farkas[3], Nasser Haddjeri[1]

[1]Stem Cell and Brain Research Institute INSERM U1208, [2]St. John's University, [3]Gedeon Richter Plc, [4]Allergan

Aim: Cariprazine, a dopamine D3-preferring D3/D2 receptor partial agonist, is FDA approved for the treatment of schizophrenia and acute manic or mixed episodes of bipolar disorder. This study used in vivo electrophysiological techniques in anesthetized rats to determine cariprazine's effect on dopaminergic cell activity in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc). Methods: Extracellular recordings of individual dopaminergic neurons were performed after oral or intravenous administration of cariprazine, the D3 receptor antagonist SB 277011A, the D2 receptor antagonist L741,626, and/or the D3 receptor agonist PD 128,907. Results: Acute oral treatment with cariprazine significantly increased and chronic cariprazine significantly decreased the number of spontaneously firing dopaminergic neurons in the VTA, but not in the SNc. Intravenous administration of cariprazine partially but significantly inhibited dopaminergic neuronal firing in both regions, which was prevented by L741,626 but not SB 277011A. In both VTA and SNc, cariprazine, SB 277011A, and L741,626 significantly antagonized the suppression of dopamine cell firing elicited by PD 128,907.

Conclusions: Cariprazine significantly modulates the number of spontaneously active VTA dopamine neurons and moderately suppresses midbrain dopamine neuronal activity. The contribution of dopamine D2 receptors to cariprazine's in vivo effects is prevalent and that of D3 receptors is less apparent.

4-C-17 The 22q11.2 deletion impairs dopamine function in the striatum: A combined clinical and preclinical study

Céline Devroye[1], Maria Rogdaki[2], Mariasole Ciampoli[2], Mattia Veronese[2], Abhishek Ashok[2], Robert McCutcheon[2], Sameer Jauhar[2], Ilaria Bonoldi[2], Maria Gudbrandsen[2], Samuel Chawner[2], Therese van Amelsvoort[2], Marianne Van Den Bree[2], Michael Owen[2], Federico Tur
[1]Istituto Italiano di Tecnologie di Genova, [2]Not listed, [3]Istituto Italiano di Tecnologia

The pathoetiology of schizophrenia is classically related to a dopamine (DA) dysregulation. Specifically, patients diagnosed with this neuropsychiatric disorder show a striatal DA dysfunction that precedes the onset of the disease, and correlates with the transition to psychosis and with the severity of symptoms. In this regard, the 22q11.2 deletion, currently considered as one of the highest genetic-based vulnerability factor for schizophrenia, may help to delineate the dopaminergic alterations associated with the transition to symptomatic stage. Thus, the present work aimed at studying the impact of 22q11.2 variation on striatal DA function and its possible association with the risk and severity of psychosis. To this purpose, a PET scan was performed to measure DA synthesis capacity (DSC) in antipsychotic-naive patients bearing the 22q11.2 deletion or duplication and healthy controls, and sub-clinical psychotic-like symptoms were evaluated. In parallel, we assessed DA markers and sensitivity in a murine model of 22q11.2 deletion (LgDel/+), using High-Pressure Liquid Chromatography and amphetamine sensitization test, respectively. Patients with 22q11.2 deletion had a higher DSC as compared to healthy controls, whereas 22q11.2 duplication carriers showed the lowest levels. These variations correlated with the psychosis risk and the severity of symptoms. Also, LgDel/+ mice displayed various alterations in DA markers, including metabolism enzymes and products, and an increased behavioural and biochemical response to amphetamine sensitization, relative to wild-type animals. Altogether, our findings demonstrate that specific dopamine alterations predate the clinical high-risk phase in patients with 22q11.2DS.

4-C-57 Disynaptic cerebellar modulation of the prefrontal cortex via the ventral tegmental area

Jorge Vera[1], Maritza Onate[1], Christopher Chen[2], Noelie Cayla[1], Kamran Khodakhah[1]
[1]Albert Einstein College of Medicine, Harvard University

The cerebellum (Cb) has been associated with cognitive disorders related to dopamine (DA) dysregulation in medial prefrontal cortex (mPFC), such as schizophrenia and autism. However, how the cerebellum contributes to DA signals in the mPFC remains to be established. Cortical DA is provided by dopaminergic neurons from the ventral tegmental area (VTA), a key region of the brain reward system. We have shown that the Cb sends direct excitatory projections to the VTA, suggesting that the Cb could contribute directly to changes in the mPFC DA levels. Here we describe experiments aimed at delineating the anatomical and functional properties of the Cb->VTA->mPFC circuit in the mouse brain. Using intersectional tracing we find that a fraction of mPFC-projecting VTA neurons receive direct synaptic inputs from the Cb, confirming a disynaptic connection. Using fiber photometry we find that

optogenetic activation of Cb inputs to VTA drives an increase in dopamine levels in the mPFC, observed as a slow build-up of DA that lasts for seconds. In contrast, DA released under the same conditions in nucleus accumbens shows a much faster kinetic (millisecond scale), suggesting that cerebellar inputs might affect these two regions differentially. Finally, we assessed the strength of this pathway to modify mPFC activity in vivo. We find that optogenetic activation of Cb inputs to VTA produces a fast and widespread excitation of mPFC single units, increasing the firing rate of ~80% of neurons with a mean latency of ~43 ms. Taken together, our data show that cerebellar activity, via VTA, can modulate DA levels and firing of neurons in mPFC at different time scales. This pathway may contribute to a cognitive role of the cerebellum, emerging as a potential link between cerebellar dysfunction and

D - Dopamine, Parkinson's Disease and neurodegeneration

4-D-18 Illuminating dopamine dynamics in Huntington's disease

Sarah Yang[1], Markita Landry[1], David Schaffer[1]
[1]University of California, Berkeley

Dysregulation of dopamine transmission plays a key role in multiple neurodegenerative diseases. In Huntington's Disease (HD) this dysregulation is thought to be biphasic, with increases in dopamine levels accompanying early chorea and decreases accompanying late akinesia. While several treatments for physical and psychiatric HD symptoms target dopaminergic neuromodulation, little is known about the relationship between dopamine and the principal cause of HD, production of mutant huntingtin protein. This lack of understanding is partly due to limited capability to visualize dopamine dynamics at the spatiotemporal resolution of both neuromodulator release (ms) and boutons (µm). Prior studies measuring bulk-averages of dopamine release suggest that evoked release in the dorsal lateral striatum decreases in R6/2 HD mice and coincides with motor symptom onset. However, knowledge of what drives decreased dopamine release is uncertain and could encompass decreased dopamine release sites, decreased dopamine quantal release per site, or a combination of the two. Herein, we utilize a near Infrared Catecholamine nanosensor (nIRCat), to image "hot spots" of dopamine activity in the striatum of R6/2 HD mice and find that late-disease decreases in evoked dopamine release are primarily driven by decreases in the number of hot spots as opposed to decreasing dopamine release quanta. These findings motivate investigation into how dopaminergic projections are affected by mutant huntingtin and whether specific targeting of these loci is important for developing gene and cell therapy efforts.

4-D-19 Assessing Vps35 gain v.s. loss of function with respect to LRRK2 activity and DA signaling

Mengfei Bu[1], Jordan Follette[2], Igor Tartarnikov[3], Shannon Wall[1], Dylan Guenther[1], Jesse Fox[3], Austen Milnerwood[4], Mark Moehle[1], Habibeh Khosbhouei[1], Matthew Farrer[1]
[1]University of Florida, [2]Univeristy of Florida, [3]University of British Columbia, [4]McGill University

VPS35 is an essential component of the retromer complex required for endosomal sorting and recycling. A mutation in VPS35 (p.D620N) leads to autosomal dominant Parkinson's disease (PD), whereas a reduction in VPS35 expression is associated with Alzheimer's dementia. Mutations in LRRK2 that constitutively increase its kinase activity are also a leading genetic cause of PD, and LRRK2 and VPS35 may directly interact. To better understand the relationship between retromer and Lrrk2, and to assess gain- versus loss-of-function, we have examined dopamine physiology and Lrrk2 kinase activity in Vps35 p.D620N knock-in (VKI) and haploinsufficient Vps35 mice (hVKO). Although there is no significant change

to the pace-making activity of the dopaminergic soma, evoked striatal dopamine release is significantly enhanced in 3-month-old VKI. Lrrk2 kinase activity as reflected by downstream Rabs phosphorylation is also constitutively elevated. In vivo treatment of VKI mice with the Lrrk2 kinase inhibitor, Mli2, normalizes both dopamine transporter (Dat) expression and striatal dopamine release. Interestingly, hVKO mice also exhibit a significant increase in evoked dopamine release with no overt change in pace-making activity. In contrast to VKI, Lrrk2 activity in hVKO mice is reduced compared to WT littermates. Together, our results suggest Vps35 p.D620N and Vps35 haploinsufficiency may result in a similar loss-of-function with Dat trafficking. Both hyper- and hypoactivation of LRRK2 is associated with retromer induced dopamine dysregulation, and these observations should be reconciled before LRRK2 kinase inhibitors are recommended for therapeutic use. Defects in vesicular trafficking may disproportionally affect dopaminergic neurons due to their extensively arborized axons and requirement for nigrostriatal axonal trafficking. Cellular context is important and is to be fully explored.

4-D-20 Peripheral administration of the Class-IIa HDAC inhibitor MC1568 partially protects against nigrostriatal degeneration in the striatal 6-OHDA rat model of Parkinson?s disease

Martina Mazzocchi[1], Susan Goulding[1], Noelia Morale-Prieto[1], Tara Foley[1], Louise Collins[1], Aideen Sullivan[1], Gerard O'Keeffe[1]
[1]University College Cork

Parkinson's disease (PD) is a neurodegenerative disorder characterized by nigrostriatal dopaminergic (DA) degeneration. There is a critical need for neuroprotective therapies, particularly those that do not require direct intracranial administration. Small molecule inhibitors of histone deacetylases (HDAC) (HDIs) are neuroprotective in in vitro and in vivo models of PD. We show that 6-hydroxydopamine (6-OHDA) treatment induces protein kinase C (PKC)-dependent nuclear accumulation of Class IIa HDAC5 in SH-SY5Y cells and cultured DA neurons. Treatment of these cultures with the Class-IIa specific HDI, MC1568, partially protected against 6-OHDA- induced cell death. In the intrastriatal 6-OHDA lesion in vivo rat model of PD, MC1568 treatment (0.5 mg/kg i.p.) for 7 days reduced forelimb akinesia and partially protected nigral DA neurons and their striatal terminals. MC1568 prevented 6-OHDA-induced increases in microglia in the striatum and nigra, and in nuclear HDAC5 levels in nigral DA neurons. These data rationalize the study of peripheral administration of Class-IIa specific HDIs as a potential neuroprotective therapy for PD.

4-D-21 Phosphodiesterase 2A : functional role in the striatum and potentially new therapeutic target in Parkinson's disease

Ségolène Bompierre[1], Cédric Yapo[1], Liliana Castro[1], Pierre Vincent[1]
[1]Sorbonne University, Insitut de Biologie Paris Seine

Aims: In Parkinson's disease (PD), the degeneration of dopaminergic neurons results in a deficit of dopamine. This situation is commonly treated pharmacologically by the administration of L-DOPA, a precursor of dopamine. However, after about 10 years of treatment, 80% of patients develop L-DOPA-induced dyskinesia (LID). In the dopamine depleted striatum, D1 type medium-sized spiny neurons (D1 MSN) become hyper-responsive to the stimulation of type 1 dopamine receptors. This hypersensitivity leads to an over-activation of the cAMP/PKA signaling pathway, resulting in the progressive development of LID. Our aim is to evaluate the potential of phosphodiesterase 2A (PDE2A), which degrades cAMP, to reduce D1 MSN hypersensitivity associated with LID. Because of its low affinity for cAMP, the stimulation of PDE2A activity through the nitric oxide (NO)/cGMP pathway could reduce

excessive cAMP levels while preserving proper responses. Methods: Biosensor imaging reports the dynamics of cAMP/PKA signaling in MSNs in striatal brain slices from young mice, or adult mice in PD and dyskinetic situation. Results: In PD and dyskinetic mouse model, D1 MSN display a larger cAMP response to transient dopamine compared to normal mice. The larger cAMP response is similar to the response measured in immature brain. Interestingly, PDE2A activation by the NO/cGMP pathway efficiently reduces the amplitude of the dopamine response in PD and dyskinetic mouse model. Conclusion: the stimulation of PDE2A activity moderates excessive cAMP levels in the response to dopamine in dyskinetic mice. These results highlight the therapeutic potential of PDE2A stimulation in the treatment of LID.

4-D-22 Investigating a dysregulated immune response in the gut underlying early PD symptoms in a model of prodromal Parkinson's disease

Sherilyn Junelle Recinto[1], Hicham Bessaiah[1], Alexandra Kazanova[1], Brendan Cordeiro[1], Christina Gavino[1], Sriparna Mukherjee[2], Jessica Pei[1], Adam MacDonald[1], Michel Desjardins[2], Louis-Eric Trudeau[2], Samantha Gruenheid[1], Jo Anne Stratton[1]

[1]McGill University, [2]Université de Montréal

Affecting approximately 9 million people worldwide, Parkinson?s disease (PD) is characterized by Lewy body pathology and a progressive loss of dopaminergic neurons in the substantia nigra pars compacta of the brain, leading to motor impairments. Decades prior to clinical diagnosis, PD patients often already manifest various autonomic dysfunctions, such as constipation. This is consistent from histopathological clinical studies indicating presence of Lewy body in the gut of about 95% of PD patients. In addition, people suffering from gut infections as well as chronic intestinal inflammation have an increased risk of developing PD. These findings substantiate the widely known Braak hypothesis suggesting a microbial infection of the gastrointestinal (GI) tract first results in peripheral neuronal damage, engendering early GI-related symptoms, and which Lewy pathology only later propagates to the brain. However, little is known about the mechanisms at play during the evolution of disease partly due to the lack of PD models able to recapitulate the protracted nature of neurodegeneration originating in the gut. To this end, we aim to extend the characterization of a mouse model, where our collaborators showed that intestinal infection triggers PD-like motor impairment in PTEN-induced kinase 1 (Pink1)-deficient mice. Our preliminary data suggests that Pink1-deficient mice, prior to onset of motor symptoms and following gut infection, displayed constipation and excessive intestinal inflammation pointing to a dysregulation in the innate immune response, as drivers of early disease. Whether these observations are mechanistically related and result in the eventual loss of dopaminergic neurons in the gut are currently under investigation.

4-D-23 Quantification of cell types in human midbrain organoids using a flow cytometry antibody panel

Rhalena Thomas[1], Julien Sirois[2], Alex Gestin[3], Shuming Li[1], Meghna Mathur[1], Thomas Durcan[1], Edward Fon[1]

[1]McGill University, [2]McGill, [3]Universite Paris-Saclay

Identification and quantification of cell phenotypes in the human brain or 3D tissue models is challenging on several levels. Histology analysis requires tissue processing and can only make use of a few selective markers at a time. Single cell RNA sequencing is expensive and can only be used on small sub-samples. Flow cytometry (FC) is an economical, efficient, and exquisitely quantitative way to

measure protein expression in samples with multiple cell types. Traditionally, FC experiments utilize only 2-4 markers, selected to define a single cell type. To identify many different cell phenotypes within a mixed cell population, more surface protein markers and advanced computational methods for analyzing FC data are needed. We quantify cell phenotypes within human midbrain organoids (hMOs), a human brain tissue model derived from induced pluripotent stem cells. The hMOs contain dopaminergic, excitatory and inhibitory neurons, astrocytes, oligodendrocytes, stem cells and differentiating cells. We tested a panel of 13 antibodies commonly used in FC and identified brain cell types within the organoids. We created a comprehensive workflow to process FC data to quantify cell phenotypes. Our pipeline contains scripts to transform and align multiple datasets, optimize unsupervised clustering and cell type annotation, quantify cell types and compare cells across conditions. Our analysis framework can be adapted to other antibody combinations and tissues providing a method to reproducibly identify cell phenotypes across FC datasets.

4-D-24 Novel biomarkers for the early identification of Parkinson's disease

Victoria Soto Linan[1], C. Gora[1], M. Peralta[2], V. Pernet[3], N. Dupré[4], P. Ruzza[5], F. Raymond[6], M. Hébert[1], M. Lévesque[1]

[1]Laval University, CERVO Brain Research Center, [2]CERVO Brain Research Center, [3]Laval University, CHU de Québec-Université Laval, [4]CHU de Québec-Université Laval, [5]Université de Padova CNR, Istituto di Chimica Biomolecolare, [6]Laval University, INAF

Parkinson's (PD) is correlated with Lewy body inclusions and the deterioration of nigral dopaminergic neurons. The diagnosis relies on motor dysfunction caused by severe neurodegeneration, despite non-motor symptomatology being present decades prior. This central lesion is hypothesized to sustain neuroinflammation, dysbiosis, and compensation mechanisms in the periphery. Given the need for efficient biomarkers for an early diagnosis, the current project aims to identify non-invasive techniques --the ERG and oral microbiota-- to detect emerging peripheral effects reflecting early central dysfunction. We first used a progressive PD mouse model, homozygous for the overexpression of human A53T variant α-synuclein (M83 mice). They underwent behavioral tests, oral microbiota swab collection, and ERG measurements bimonthly for eight months. Histological analyses of the brain, optic nerve, and retinal tissue are then processed. Oral microbiota is quantified by 16S sequencing. Next, early diagnosed PD patients and age-matched controls were recruited, including both genders. They also underwent ERG testing, oral bacteria Salivette swabs, and an unstimulated saliva collection for complementary proteomic/metabolomic analysis. Our results in mice and humans showed that retinal ganglion cell function impairment is an early retinal change in PD. Dysbiosis in the oral microbiota showed key bacterial changes that support the role of opportunistic pathogens in pathology. Altogether, the current project reveals non-invasive tools to indirectly track central neurological changes, helping us detect Parkinson's early and providing a wider window for neuroprotective therapeutic intervention.

4-D-25 Differential co-expression of tyrosine hydroxylase and vesicular glutamate transporter 2 in human and rodent aging and a rotenone model of Parkinson's disease

Silas Buck[1], Briana De Miranda[1], J. Timothy Greenamyre[1], Kenneth Fish[1], Jill Glausier[1], David Lewis[1], Zachary Freyberg[1]

[1]University of Pittsburgh

Selective degeneration of dopamine (DA) neurons is observed in both aging and Parkinson's disease (PD), with the substantia nigra pars compacta (SNc) showing greater vulnerability than the ventral

tegmental area (VTA). This regional difference may be related to expression of the vesicular glutamate transporter 2 (VGLUT2). Indeed, we find that DA neurons that survive neurodegeneration show VGLUT2 upregulation. While the protective impact of VGLUT2 expression in DA neurons has been described across several PD experimental models, changes in DA neuron VGLUT2 expression across healthy aging have not been investigated in any animal or human models. In this study, we sought to compare DA neuron VGLUT2 expression between healthy aging and a rotenone model of DA neurodegeneration. First, we examined DA neuron VGLUT2 expression in young, middle-aged and aged postmortem human brains via multiplexed RNAscope imaging of tyrosine hydroxylase (TH) and VGLUT2 mRNA. We found comparable TH and TH/VGLUT2 cell loss with age in the VTA and SNc, albeit with greater vulnerability in the SNc relative to the VTA. In mice, a loss of TH ($p < 0.05$) and TH/VGLUT2 ($p < 0.001$) cells was observed with age as well. In contrast, we observed that rotenone caused a significant loss of TH cells ($p < 0.001$), while TH neurons co-expressing VGLUT2 were more resilient ($p > 0.05$). In conclusion, the DA neuron VGLUT2 expression may play an important role in vulnerability and resilience to neurodegeneration in response to neurotoxic insult but not in the course of healthy aging.

4-D-26 Involvement of autophagy in L-Dopa-induced dyskinesia

Carina Plewnia[1], Michael Feyder[1], Ori Liebermann[2], Giada Spigolon[1], Alessandro Piccin[1], Lidia Urbina[1], Benjamin Dehay[3], Qin Li[4], Per Nilsson[1], Mikael Altun[1], Emanuela Santini[1], David Sulzer[2], Erwan Bezard[3], Anders Borgkvist[1], Gilberto Fisone[1]

[1]Karolinska Institutet, [2]Columbia University, [3]Bordeaux University, [4]Motac Neuroscience Ltd

Autophagy plays an essential role in maintaining effective turnover of proteins and damaged organelles. It is intensively studied in cancer, metabolic and neurodegenerative diseases, but little is known about its role in pathological conditions linked to altered neurotransmission. Here, we examined the involvement of autophagy in L-Dopa-induced dyskinesia (LID), a motor complication developed by Parkinson's disease (PD) patients in response to dopamine replacement therapy. In PD, the loss of dopamine in the dorsal striatum leads to the sensitization of dopamine D1 receptors (D1R) which confers on L-Dopa the ability to activate the mammalian target of rapamycin complex 1 (mTORC1), which promotes protein synthesis and reduces autophagy. Rapamycin, an inhibitor of mTORC1 counteracts LID, but virtually nothing is known about the impact on LID of dysregulated autophagy. We used a mouse and a non-human primate model of PD to examine changes in autophagy associated with chronic L-Dopa administration. We found that LID is associated with accumulation of the autophagy-specific substrate p62, indicating autophagy deficiency. Increased p62 was observed in the striatal projection neurons of the direct pathway. Inhibition of mTORC1 with rapamycin counteracted the impairment of autophagy produced by L-Dopa and reduced LID. Importantly, the anti-dyskinetic effect of rapamycin was lost when autophagy was constitutively suppressed in D1R-expressing striatal neurons, through inactivation of the autophagy-related gene protein 7. These findings indicate that, in PD, augmented responsiveness at D1R leads to dysregulated autophagy, and results in the emergence of LID. They further suggest the enhancement of autophagy as a therapeutic strategy against dyskinesia.

4-D-27 Neuroimaging VMAT2 in Parkinson's Disease with Rapid Eye Movement Sleep Behaviour Disorder

Mikaeel Valli[1], Sang Soo Cho[2], Carme Uribe[1], Mario Masellis[3], Robert Chen[4], Alexander Mihaescu[1], Antonio Strafella[1]

[1]CAMH, [2]Seoul National University, [3]Sunnybrook Health Sciences Centre, [4]Toronto Western Hospital

REM sleep behaviour disorder (RBD) is a common condition found in 50% of Parkinson's disease (PD) patients. Molecular imaging evidence shows that PD with RBD (PD-RBD+) show lower dopamine transporter activity within the caudate and putamen compared to PD without RBD (PD-RBD-). However, the characterization of the vesicular monoamine transporter 2 (VMAT2), an index of nigrostriatal dopamine innervation, has been rarely explored in PD patients with RBD. Thus, we enrolled 15 PD-RBD+, 15 PD-RBD- and 15 age matched healthy controls (HC) for the [11C]DTBZ PET imaging study. This technique measures VMAT2 availability within striatal regions of interest (ROI). Mixed effect model was used to compare the radioligand binding of VMAT2 between the three groups for each striatal ROI, while co-varying for sex, cognitive and depression scores. Multiple regressions were also computed to predict clinical measures from group condition and VMAT2 binding within all ROIs explored. We observed significant main effect of group condition on VMAT2 availability within the caudate, putamen, ventral striatum, globus pallidus, substantia nigra, and subthalamus. Specifically, we observed that both PD-RBD+ and PD-RBD- group had lower VMAT2 availability compared to HC. Only PD-RBD- showed a negative relationship between motor severity and VMAT2 availability within the left caudate. Our findings reveal that both PD patient subgroups had similar denervation within the nigrostriatal pathway. This study was unable to detect interactions between clinical scores and radioligand binding in PD-RBD+ patients. In sum, VMAT2 and striatal dopamine denervation in general may not be a significant contributor to driving RBD pathology in PD patients (Valli et al., 2021, Mol Brain).

E – Development and diversity of the dopamine systems

4-E-28 Molecular profiling of GABAergic neuron subtypes in the developing ventral midbrain

Özge Düdükcü[1], Divya Raj[1], Lieke van de Haar[1], Louisa Linders[1], Nicky van Kronenburg[1], Mark Broekhoven[1], Youri Adolfs[1], Frank Meye[1], R. Jeroen Pasterkamp[1]
[1]University Medical Center Utrecht, Utrecht University

Dopaminergic neurons in the ventral midbrain (mDA) are surrounded by various GABA-rich nuclei. While a small subset of GABA neurons is located in the mDA neuron pool, most GABAergic neurons are localized in surrounding GABA-rich nuclei, such as the substantia nigra pars reticulata (SNr) and interpeduncular nucleus (IPN). This organization is important since mDA neurons have specific dendritic projections into adjacent regions. For example, mDA neurons in the SNc project dendrites into SNr, while avoiding the IPN. The full extent of GABAergic neuron subtypes present in the ventral midbrain and the mechanisms that underlie the development and function of these subtypes are largely unknown. Therefore, we used single cell RNA sequencing (scRNAseq) of isolated GABAergic neurons (expressing VGAT) in the developing mouse ventral midbrain. We identify 19 GABAergic clusters and define distinct GABAergic subtypes based on transcriptomic profiles. These results were validated by immunohistological localization of selected markers and used to generate a spatial map within the ventral midbrain. Interestingly, some of the GABAergic clusters express dopaminergic markers. To characterize these subtypes, we performed electrophysiology and axon tracing. Further, based on the scRNAseq data, we show that Netrin-1 dictates the positioning of different GABAergic subtypes via distinct receptor mechanisms. Overall, our work provides novel insight into the organization of GABAergic neuron subtypes in the ventral midbrain and begins to dissect the mechanisms that underlie their development and function.

4-E-29 Regional analysis of dopaminergic neurons reveals subsets with high steady-state activation of the integrated stress response

Elana Lockshin[1], Nicole Calakos[1]
[1]Duke University

The integrated stress response (ISR) is a highly conserved biochemical pathway that is conditionally, and typically transiently, activated by any of four eIF2alpha kinases to markedly change protein synthesis. The ISR is widely known for its proteostasis role in responding to diverse cell stressors, but also has distinct roles in the brain. In the brain, the ISR influences the induction of long-term synaptic plasticity and modifies learning thresholds for instantiation of long-lasting memory. We recently found that a class of neuromodulatory cells, striatal cholinergic interneurons, were unusual in that they uniformly showed ISR activation under normal, steady-state conditions. In dopaminergic neurons, rather than uniform ISR activation, we found a broad range of ISR states. Here, we perform a regional subanalysis to determine whether ISR activation in a fraction of dopamine neurons is randomly distributed, or corresponds to anatomically defined subsets with the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). Quantitative immunohistochemical analysis of phospho-PERK levels shows that medial SNc levels were significantly higher as compared to lateral SNc and VTA. Ongoing studies are evaluating sex effects and concordance across ISR state measures. Interestingly, this medial region of the SNc is the last to degenerate in Parkinson's disease, potentially implicating a protective role of higher ISR tone in these cells.

4-E-30 Axon-derived netrin-1 regulates midbrain GABAergic migration and substantia nigra development

Divya Darwin Arulseeli[1], Sara Brignani[1], Ewoud Schmidt[1], Ozge Dudukcu[1], Laurens Grossouw[1], Youri Adolfs[1], Juan Moreno-Bravo[2], Alain Chedotal[2], Jeroen Pasterkamp[1]
[1]Utrecht University, [2]Sorbonne Université

Cell migration in the central nervous system is shown to be regulated by guidance cues produced locally by cells residing at choice points. We show a hitherto unknown role for Netrin-1 in the migration of dopaminergic neurons and positioning of GABAergic neurons for the formation of Substantia nigra pars reticulata (SNr). Mice lacking Netrin-1 protein show increased lateral-ventral migration of dopaminergic neurons of the compacta (SNc). Normally, SNc neurons project their dendrites ventrally into the GABA-rich SNr. In Netrin-1 KO mice, GABAergic neurons of the anterior SNr fail to migrate ventrally to form this brain nucleus. Intriguingly, conditional ablation of Netrin-1 from local cellular sources in the midbrain does not mimic the defects in cellular migration seen in the complete knockout. Alternatively, we find that Netrin-1 derived from forebrain neuronal axons affects migration and positioning of neurons in the SNc/SNr in the ventral midbrain. We also identify the possible Netrin-1 signaling receptor that mediates these effects of Netrin-1. This study signifies the role of axons as "carriers" of guidance cues to affect cellular organization and connectivity in the brain.

H – Dopamine drug development and pharmacology

4-H-31 Novel dual-target mu opioid (MOR) and dopamine D3 receptors (D3R) ligands as potential non-addictive pharmacotherapeutics for pain management

Alessandro Bonifazi[1], Francisco Battiti[1], Elizabeth Saab[1], Julie Sanchez[2], Saheem Zaidi[3], Vsevolod Katritch[3], Meritxell Canals[2], Robert Lane[2], Amy Newman[1]
[1]National Institutes of Health, [2]University of Nottingham, [3]University of Southern California

The need for safer pain-management therapies with decreased abuse liability inspired a novel drug design that retains mu-opioid receptor (MOR)-mediated analgesia, while minimizing addictive liability. We recently demonstrated that targeting dopamine D3 receptors (D3R) with highly selective antagonists/partial agonists can reduce opioid self-administration and reinstatement to drug seeking in rodent models without diminishing antinociceptive effects. The identification of D3R as a target for the treatment of opioid use disorders, prompted the idea of generating a class of ligands presenting bitopic or bivalent structures, allowing the dual-target binding of MOR and D3R. Structure-activity relationship studies using computationally aided drug-design, physical-chemical multiparameter optimization score calculations predicting compounds ability to cross the blood brain barrier and target the central nervous system, and in vitro binding assays led to the identification of potent dual-target leads, based on different structural templates, with moderate (sub-micromolar) to high (low nanomolar/sub-nanomolar) binding affinities and receptor subtypes selectivity. BRET-based functional studies confirmed the desired MOR agonist-D3R antagonist/partial agonist efficacies, suggesting potential for maintaining analgesia with reduced opioid-abuse liability.

4-H-32 Discovery and characterization of a functionally selective ghrelin receptor ligand for modulating brain dopamine homeostasis

Joshua Gross[1], David Kim[2], Yang Zhou[1], Dan Jansen[2], William Wetsel[1], Marc Ferrer[2], Krisztian Toth[3], Juan Marugan[2], Marc Caron[1], Lawrence Barak[1]

[1]Duke University, [2]National Center for Advancing Translational Sciences (NCATS), [3]Campbell University

The growth hormone secretagogue receptor-1a (GHSR1a) is the cognate G protein-coupled receptor (GPCR) for the hormone ghrelin. The GHSR1a modulates brain dopamine (DA) homeostasis and is neuroprotective within DA neurocircuits. GHSR1a-mediated signaling originates from pharmacologically separable G protein- and β-arrestin (βarr)-dependent pathways and consequently, GHSR1a-dependent physiological responses rely upon their distinctive signaling contributions. Thus, when treating disorders of disrupted DA homeostasis, a pharmacological strategy that modulates biased GHSR1a signaling may uncouple desired therapeutic outcomes from unwanted side effects. By high-throughput screening of ~47,000 small molecules, we discovered a GHSR1a-selective, G protein-biased agonist -- N8279 (NCATS-SM8864) -- based on a novel chemotype. Comprehensive pharmacological characterization reveals that N8279 elicits potent and biased Gαq activity at both the apo- and ghrelin-bound GHSR1a. Further biochemical analysis and molecular modeling demonstrate that N8279 signaling requires sites within the extracellular domain of the GHSR1a, especially extracellular loop 2 (ECL2). Collectively, our findings support that N8279 possesses a bitopic, extended binding mode into the GHSR1a ECD that preferentially favors Gαq signaling over alternative G proteins (Gαi/o, Gα12/13) and βarr2-dependent cellular responses. Critically, N8279 is brain penetrant in mice and attenuates dysfunctional DA-mediated behaviors in both genetic and pharmacological mouse models of hyperdopaminergia. Our findings provide insight into mechanisms governing GPCR signaling and illustrate how functional selectivity can be leveraged to develop GHSR1a pharmacotherapeutics that normalize pathological disruptions of brain DA homeostasis.

4-H-33 D-neuron (trace amine neuron, type 1), TAAR1 ligand neuron: A clue for medicinal chemistry

Keiko Ikemoto[1]

[1]Iwaki City Medical Center

Due to complexity of the human central nervous system (CNS), there is an urgent necessity to elucidate pathophysiology of disorders in the human CNS. I specified anatomical subgroups of human CNS D-neurons (aromatic L-amino acid decarboxylase (AADC)-only neuron = dopa decarboxylating neurons = trace amine (TA) neurons, type 1) (Ikemoto 2016), demonstrated D-neuron decrease in the nucleus accumbens (Acc, D16) of postmortem brains with schizophrenia (Ikemoto et al. 2003), and established "D-cell hypothesis (TA hypothesis) of schizophrenia" (Ikemoto 2012). The human D-neuron system is far developed in the forebrain in comparison with that of other species, including non-human primates (Kitahama et al. 2009). The TAAR1 (TA-associated receptor 1), exclusive receptor of TAs in humans, has a large number of ligands including tyramine, â-phenylethylamine and methamphetamine, which affect on human mental states. The "D-cell hypothesis" is that accumbal D-neuron decrease in schizophrenia and consequent TAAR1 stimulation decrease to terminals of midbrain ventral tegmental area (VTA) DA neurons induces mesolimbic DA hyperactivity (Bradaia et al. 2009) of schizophrenia. Dysfunction of subventricular neural stem cells (NSC) located in Acc (Sanai et al. 2004) is the cause of D-neuron decrease in Acc (Ikemoto 2012). "D-cell hypothesis", linking DA dysfunction hypothesis to NSC dysfunction hypothesis, explains mechanisms of mesolimbic DA hyperactivity and disease progression of schizophrenia, and predicts effectiveness of TAAR1 agonists or TAAR1 partial agonists (Revel et al. 2013), though modulation by serotonin mechanisms further be involved.

4-H-34 The novel atypical antipsychotic cariprazine demonstrates dopamine D2 receptor-dependent partial agonist actions on rat mesencephalic dopamine neuronal activity

Sarah Delcourte[1], Charles Ashby Jr[2], Renaud Rovera[1], Béla Kiss[3], Nika Adham[4], Bence Farkas[3], Nasser Haddjeri[1]

[1]Stem Cell and Brain Research Institute INSERM U1208, [2]St. John's University, [3]Gedeon Richter Plc, [4]Allergan

Aim: Cariprazine, a dopamine D3-preferring D3/D2 receptor partial agonist, is FDA approved for the treatment of schizophrenia and acute manic or mixed episodes of bipolar disorder. This study used in vivo electrophysiological techniques in anesthetized rats to determine cariprazine's effect on dopaminergic cell activity in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc). Methods: Extracellular recordings of individual dopaminergic neurons were performed after oral or intravenous administration of cariprazine, the D3 receptor antagonist SB 277011A, the D2 receptor antagonist L741,626, and/or the D3 receptor agonist PD 128,907. Results: Acute oral treatment with cariprazine significantly increased and chronic cariprazine significantly decreased the number of spontaneously firing dopaminergic neurons in the VTA, but not in the SNc. Intravenous administration of cariprazine partially but significantly inhibited dopaminergic neuronal firing in both regions, which was prevented by L741,626 but not SB 277011A. In both VTA and SNc, cariprazine, SB 277011A, and L741,626 significantly antagonized the suppression of dopamine cell firing elicited by PD 128,907. Conclusions: Cariprazine significantly modulates the number of spontaneously active VTA dopamine neurons and moderately suppresses midbrain dopamine neuronal activity. The contribution of dopamine D2 receptors to cariprazine's in vivo effects is prevalent and that of D3 receptors is less apparent.

I – Anatomy and physiology of Dopamine systems

4-I-35 Phase resetting in mouse substantia nigra pars compacta dopamine neurons

Matthew Higgs[1], Michael Beckstead[1]

[1]Oklahoma Medical Research Foundation

Input-output properties of pacemaker neurons can be characterized by the phase resetting curve (PRC), which represents the sensitivity of inter-spike interval (ISI) length to inputs arriving at different phases of the firing cycle. We determined PRCs of substantia nigra pars compacta dopamine (DA) neurons and local GABA neurons in mouse brain slices using gramicidin-perforated current-clamp recordings and noise stimuli. DA neuron PRCs were of type 1, meaning depolarizing inputs at all phases shortened the ISI, and most had a broad body peaking at early or late phase followed by a sharp peak near the end. GABA neuron PRCs were also type-1 but had larger amplitudes (indicating higher sensitivity) and different shapes. In DA neurons, current pulses applied at most phases produced membrane potential (Vm) responses that quickly relaxed toward the unperturbed Vm trajectory, leaving a small offset responsible for the change in ISI length. In contrast, GABA neurons showed less relaxation of the Vm responses to current pulses, accounting for their higher sensitivity. These observations suggest that in DA neurons, an attractive fixed point, or moving resting potential set by slowly changing state variables, exists across most of the firing cycle. The slow variables include intracellular calcium levels activating SK-type potassium channels. Although SK channels are thought to be activated primarily at early phases, the SK blocker apamin increased sensitivity across early and late phases, suggesting interaction with other slow state variables such as Kv4 inactivation. This work identifies differences in input-sensitivity between two neuron types in the SNc and suggests a possible role for DA cell SK channels in synaptic integration throughout the firing cycle.

4-I-36 Dopamine neuron axons in the corpus callosum: potential role in experience-dependent myelination

Megan Caldwell[1], Lauren Reynolds[2], Elizabeth Fiore[1], Alyssa Martin[1], Darya Ryndych[1], Dan McCloskey[1], Stephen Rayport[3], Susana Mingote[1], Cecilia Flores[4], Leora Yetnikoff[1]
[1]City University of New York, [2]Sorbonne Université, [3]Columbia University, [4]McGill University

Experience-dependent myelination is emerging as a robust form of brain plasticity. Alterations in myelin regulation impact social avoidance behaviors, motor learning and long-term retention of emotional memories. Dysregulation in large myelinated, white matter tracts such as the corpus callosum have been implicated in dopamine-related neuropsychiatric disorders, including schizophrenia and drug addiction. These findings suggest a potential role for dopamine neurons in experience-dependent myelination. To investigate a role for midbrain dopamine neurons in experience-dependent myelination of the corpus callosum, we have examined adult male DAT-cre mice injected with a cre-dependent eYFP virus into the midbrain, to visualize dopamine neuron axons in the corpus callosum. We have found dopamine axons and varicosities in the corpus callosum, with the greatest number medially. The density is substantially lower than seen in the neighboring striatum or medial prefrontal cortex. Then, using proximity ligation assay in DAT-IREScre;ChR2-EYFP mice to label dopamine vesicle clusters at putative release sites, within 20 nm of the plasmalemma, we have found a high density of putative dopamine release sites in medial aspects of the corpus callosum. We are now quantifying dopamine axon architecture in the corpus callosum of mice repeatedly exposed to amphetamine to determine drug-dependent plasticity. A similar approach may be taken to address maturational changes in dopamine axon architecture in the corpus callosum, comparing adolescent and adult mice. With this approach we will begin to identify the relationship between midbrain dopamine neuron function and experience-dependent myelination.

J – Dopamine and brain circuitry

4-J-37 Awry acetylcholine-dopamine interaction within the nucleus accumbens of mice leads to impaired associative learning

Miguel Skirzewski[1], Oren Princz-Lebel[1], Alycia Crooks[1], Liliana German-Castelan[1], Gerard Kim[1], Sophie Henke Tarnow[1], Amy Reichelt[1], Miao Jing[2], Fangmiao Sun[3], Yajun Zhang[3], Yulong Li[3], Lisa Saksida[1], Vania Prado[1], Marco Prado[1], Timothy Bussey[1]

[1]Western University, [2]Pekin University, [3]Peking University

Learning to associate environmental cues and rewards is fundamental for survival. Psychiatric disorders displaying abnormal associative learning endophenotypes have been correlated with altered local network circuitry mechanisms within the nucleus accumbens (NAc). Striatal cholinergic interneurons (CINs) are the main source of acetylcholine (ACh) in the striatum and play a robust neuromodulatory role onto dopaminergic axons, glutamatergic excitatory inputs, and synaptic plasticity on spiny projecting neurons (SPNs). Moreover, CINs are considered key players in reward-related learning behaviors because during salience, neurons respond with brief pauses, flanked by bursts of increased activity. Notwithstanding, it is still poorly described how altered ACh release from CINs affects NAc dopamine (DA) function and SPN activity in mice performing a reward-based associative learning task. We combined the use of automated touchscreens to assess a classic conditioning pavlovian task, transgenic mice harboring deficits in the expression of the vesicular ACh transporter (VAChT) in CINs (D2-cre,VAChTf/f), and fiber photometry to record NAc ACh, DA, and calcium dynamics from SPNs. We show that mice with impaired ACh release from CINs leads to 1) deficits at discriminating cue stimuli predicting rewards, 2) impaired DA signal-to-noise ratio dynamics between CS+/CS- stimuli, 3) abnormal D1-SPN/D2-SPN calcium dynamics, and 4) these behavioral and NAc circuitry deficits are rescuable by bilateral expression of VAChT in NAc CINs via custom-made AAV-VAChT infection. Our findings suggest that ACh release from NAc CINs is fundamental to regulate local network circuitry underlying associative learning behaviors, yet deficits can be rescued during adulthood.

4-J-39 Neural dopamine dynamics underly the individuation of alcohol drinking behavior

Emily Teichman[1], Arthur Godino[1], Erin Calipari[2], Eric Nestler[1], Morel Carole[1], Ming-Hu Han[1]

[1]Icahn School of Medicine at Mount Sinai, [2]Vanderbilt University

Context: Innate variability in response to reward is a striking but understudied phenomenon. This heterogeneity is prominently seen among those that consume alcohol recreationally; some drink casually while others drink in an uncontrolled manner. Dopamine dynamics are critical in encoding the reinforcing properties of rewarding and drug stimuli, yet, how these dynamics contribute to the phenotypic divergence seen in drug consumers remains unknown. Aim: Here, we aim to isolate the pre-existing neural dynamics that may explain behavioral individuation in response to natural reward and thus, future propensity to consume alcohol. Methods: Following measurement of behavioral responses to natural reward, we electrophysiologically record in vivo, dopamine neurons' activity and their response to alcohol (i.v.) . We then utilize in vivo fiber photometry in freely behaving mice to record projection-specific dopamine dynamics elicited by natural rewards and investigate how voluntary alcohol drinking is predicted by these dopamine dynamics. Finally, using chemogenetics we manipulate these activity patterns and subsequently control alcohol consumption. Results: We identify that innate behavioral responses to reward are mirrored by heterogeneous yet distinct dopamine firing profiles. We then establish that individual alcohol profiles are associated with pre-existing dopamine dynamics and find that individuals with a hyperdopaminergic activity profile have a lower neuronal response to alcohol

and develop lower a lower preference for alcohol in the future. Conclusion: By assessing innate variability in response to rewards- natural and drug- this project will provide novel insights into the neural dynamics and mechanisms driving the phenotypic divergence we see among alcohol consumers.

K – Dopamine receptors, transporters & signalling

4-K-41 Pharmacological characterization of alpha-pyrrolidinovalerophenone enantiomers

Marco Niello[1], Jakob Schwazer[1], Julian Maier[1], Ronan O'Shea[2], Alexander Hoffman[2], Carl Lupica[2], Kathrin Jäntsch[1], Michael Baumann[2], Harald Sitte[1]
[1]Medical University of Vienna, [2]National Institute on Drug Abuse (NIDA)

α-pyrrolidinovalerophenone (α-PVP) is a synthetic analog of the naturally-occurring stimulant cathinone, a main psychoactive compound in the Khat plant (Catha edulis). α-PVP is manufactured in Asian laboratories and sold worldwide by internet vendors and street drug dealers. After high-dose or chronic misuse, α-PVP can cause serious adverse effects including aggression, paranoia, and seizures (EMCDDA 2016). The α-PVP formulation in the recreational marketplace is composed of two enantiomers: (R)- and (S)- α-PVP. Previous findings with the racemic α-PVP show it acts as a potent inhibitor of the dopamine and norepinephrine transporters (DAT and NET, respectively; Meltzer et al. 2006). While these findings imply a link between transporter inhibition and adverse effects of α-PVP, a thorough characterization of its enantiomers is missing. Our work combined in vitro uptake-inhibition assays, site-directed mutagenesis, in vitro whole cell patchnclamp and ex vivo fast scan cyclic voltammetry (FSCV). We found a pronounced enantioselectivity of α-PVP on both DAT and NET, but not on the serotonin transporter (SERT), organic cation transporter 2 and 3 (OCT2 and OCT3, respectively). Despite the lack of enantioselectivity on OCT2, our data show that α-PVP inhibits OCT2 with a micromolar affinity, suggesting a possible role of OCT2 in α-PVP-mediated effects. Moreover, a closer look at the interaction between α-PVP and DAT shows that the binding-mode of α-PVP may overlap with the one of cocaine. Hence, the present study provides new insight in the pharmacology of α-PVP elucidating its enantioselectivity and its pharmacological differences when compared to cocaine. Our findings may help in developing new approaches for the treatment of psychostimulant addiction.

4-K-42 D1-medium spiny neurons exhibit a temporally regulated differential dopamine sensitivity

Ainoa Konomi Pilkati[1], Thorvald Andreassen[1], Andreas Sørensen[1], Kenneth Madsen[1], Ulrik Gether[1]
[1]Copenhagen University

The basal ganglia are known to play a key role in movement, emotion, motivation, and learning. Dopaminergic neurons from the midbrain project to the striatum, the entrance of the basal ganglia circuit. The striatum is structurally and functionally separated into dorsal striatum (habit formation) and ventral striatum (goal-directed actions). Its main cell type is the medium spiny neuron (MSN), divided into dopamine (DA) D1-receptor (D1R-MSNs) and D2-receptor MSNs (D2R-MSNs). The D1R is thought to have low DA affinity (µM range) as compared to D2R (nM range). It has accordingly been assumed that D1R-MSNs sense phasic release of DA while D2R-MSNs sense lower levels of tonic DA release. Here, we study DA responsiveness of D1R-MSNs by transducing striatal primary cultures from D1-Cre mice or wild-type rats with the genetically encoded protein kinase A (PKA) sensors ExRai-AKAR2 or AKAR4. By use of live imaging, we can track PKA activity in individual neurons with high temporal and spatial resolution. Strikingly, we observe that individual D1R-MSNs show differential responsiveness to DA with a subpopulation of D1R-MSNs responding to nM DA concentrations while other D1R-MSNs require µM

concentrations. The response profile of the single neuron is generally maintained after 1h and even after 24h. However, a fraction of the neurons shifts their sensitivity, becoming either more or less sensitive towards DA. We observe moreover that overexpression of D1R in D1R-MSNs increases the fraction of hyperresponsive neurons, suggesting that differential receptor expression may contribute to the observed phenotype. In addition to reveal a so far unappreciated dynamic signaling heterogeneity of D1R-MSNs, the data challenge the classical assumption of low-affinity D1Rs and high-affinity D2Rs.

4-K-43 Systematic characterization of human dopamine transporter missense mutations from a Danish cohort of psychiatric patients

Anna Campana[1], Carmen Klein Herenbrink[1], Jonatan Fullerton Støier[1], Thomas Werge[2], Freja Herborg[1], Ulrik Gether[1]

[1]University of Copenhagen, [2]Copenhagen University Hospital

The dopamine transporter (DAT) shapes extracellular dopamine (DA) levels through high-affinity Na+-dependent reuptake of DA. Several reports suggest that missense mutations in the DAT gene (SLC6A3) are associated with neuropsychiatric diseases including ADHD, autism and bipolar disorder. DAT mutations have also been implicated in both infantile and early-onset parkinsonism. Here, we systematically characterize disease-associated DAT mutations in vitro to identify and classify mutational phenotypes. From an exome-sequenced Danish cohort of 19,005 individuals (iPSYCH2012), we chose 53 DAT mutants predominantly identified in patients with ADHD, ASD, schizophrenia or bipolar disorder. The mutants were expressed in HEK293 cells and evaluated using classical [3H]-dopamine uptake assays and a new "sniffer cell" assay exploiting T-Rex 293 cells expressing genetically encoded DA sensors. A large fraction of mutants was functionally impaired with lowered Vmax and/or increased Km values. For 14 mutations we were unable to detect any activity. Additionally, we identified two variants with enhanced uptake capacity. To further dissect the molecular phenotype, the mutants are being tested for altered surface expression/inhibitor binding using a novel, fluorescently tagged cocaine analogue, DG3-80. Moreover, by use of the sniffer cells, all mutants were screened for constitutive DA efflux, a phenotype earlier reported for disease-associated DAT mutants. Remarkably, constitutive efflux was only observed for the autism associated T356M variant that previously was shown to possess this phenotype. Summarized, our results provide an important framework for deciphering mechanisms underlying how perturbed DAT function may contribute to neuropsychiatric disease.

4-K-45 Dynamics of dopamine signal integration in striatal neurons

Pierre Vincent[1], Ségolène Bompierre[1], Cédric Yapo[1], Anu Nair[2], Elia Mota[1], Jeanette Kotaleski[3], Liliana Castro[1]

[1]Sorbonne Université - CNRS, [2]KTH Royal Institute of Technology, [3]Karolinska Institutet

Striatal Medium-sized Spiny Neurons (MSNs) integrate dopamine signals through the cAMP-PKA signaling pathway. Although the signaling enzymes involved in this integration are well identified, their respective contributions to the dynamics of signal processing remain unclear. We used biosensor imaging in mouse brain slice preparations to analyze the cAMP and PKA signals triggered by transient dopamine stimulations. In silico simulations were used to test SPN's responsiveness to various dynamic dopamine signals. D1 and D2 receptors, expressed by two separate sub-classes of MSNs, showed a similar sensitivity to dopamine. The D1 response was efficiently suppressed by cholinergic agonists activating M4 muscarinic receptors, while the D2 receptor suppressed adenosine A2A signals. PDE10A appeared as the only PDE able to decrease cAMP concentration below micromolar level, and its activity

was therefore required to deactivate PKA. PDE1B was shown to mediate glutamate - dopamine interactions, while PDE2A mediated a cross-talk between nitric oxide (NO) and dopamine. PDE2A and PDE4 appeared as modulators of peak dopamine responses. PKA-dependent phosphorylation appeared highly non-linear, probably as a result of DARPP-32-mediated inhibition of phosphatases. Overall, our data show that D1 MSNs are geared to respond in an all or none way to transient increases in dopamine. In contrast, D2 MSNs respond to transient lack of dopamine. Such dynamic description of signaling integration is required to better understand the effects of novel drugs, and define novel therapeutic strategies for diseases affecting the dopaminergic system.

M - Dopamine and behavior

4-M-46 D1 receptors in the dorsomedial striatum regulate motor coordination

Stefano Cataldi[1], Clay Lacefield[1], Gautam Kumar[1], Shashaank N[1], David Sulzer[1]
[1]Columbia University

The acquisition of motor learning requires effective dopamine (DA) neurotransmission and is damaged following the loss of DA in people with Parkinson's Disease (PD). Recent studies suggest that DA function is altered at early stages prior to DA neuronal death, indicating the presence of a prodromal phase in which intervention would be beneficial. We developed a treadmill task to track changes in mouse locomotor coordination during practice running at a range of speeds. We analyzed body position and paw movement to evaluate changes in motor coordination over practice using DeepLabCut and custom-built code. By simultaneous evaluation of motor coordination improvements and fiber photometry recordings of neuronal calcium activity during training, we found that direct pathway DMS neurons exhibited reduced activity as the mouse became proficient at running on the treadmill. In contrast, direct pathway activity in the DLS was similar throughout training and did not correlate with increased skill proficiency. Local injection of SCH-39166, a D1-antagonist, in the DMS shows delays in motor learning while there was no effect if the drug is injected in well trained animals. Injection of the same compound in the DLS appear to affect performance independently of the stage of learning. We successfully developed a novel model to study motor learning. Our data suggest that DA contribution to the direct pathway, via D1 receptors, is necessary for accurate acquisition and performance of a motor task. These experiments are crucial for the understanding of these systems in both healthy and PD brain, to help develop more effective therapies, and to identify individuals at risk of PD, allowing application for protective strategies.

4-M-47 Functional characterization of genetically defined subtypes of nigrostriatal dopamine neurons

Maite Azcorra[1], Rajeshwar Awatramani[1], Daniel Dombeck[1]
[1]Northwestern University

Recent single cell studies have identified several molecularly distinct subtypes of midbrain dopamine (DA) neurons (Poulin et al. 2016), with different cell body locations, projection patterns, and vulnerability in PD models. But whether this genetic division relates to the function of the neurons remains unanswered. Here, we characterized the activity of many of these subtypes in the mouse during behaviors that involve DA signaling, such as locomotion, reward acquisition/consumption, response to aversive stimuli, and Pavlovian conditioning. We used intersectional genetic strategies to label the individual DA subtypes with the calcium indicator GCaMP6f, and recorded changes in calcium signaling using one-photon fiber photometry. We found that, beyond the previously identified differences in

projection patterns, nigrostriatal DA subtypes have distinct functional signaling patterns that can be dissociated by their molecular fingerprint. These results could explain previous incongruencies in DA signaling patterns found during recordings of undefined DA neuron subtypes (Howe et al. 2016, Dodson et al. 2016, Da Silva et al. 2016 Coddington et al. 2018), and have implications for the research and treatment of DA related disorders.

4-M-48 Stress influences on the explore-exploit tradeoff in reward-guided decision making

Dana Mueller[1], Cathy Chen[1], Anila Bano[1], Elinor Wood[1], Nicola Grissom[1]
[1]University of Minnesota

Stress has been implicated in changes in decision making, but the implementation of such changes via computational processes of reinforcement learning remains unclear. Stress causes a widespread refocusing of cognitive resources, and alters catecholamine function, including dopamine, to affect reward valuation. We examined the effects of acute saline injection stress as part of a behavioral pharmacology experiment on decision making in mice in a restless bandit task. This translational task involves repeatedly selecting between two illuminated squares on a touchscreen operant chamber, which are rewarded probabilistically. This bandit task is "restless" because the reward probability of each choice changes randomly and independently across trials. To maximize reward, animals have to balance between exploiting a current high-value option and exploring potential better alternatives. We hypothesized that acute stress would increase reward sensitivity, leading mice to exploit more choices following rewarded trials (win-stay). Thirty-two 129/b6j F1 mice (16 male and 16 female) were tested on restless bandit schedules with and without saline injection prior to testing in a within-subjects design. Our preliminary results indicate that acute injection stress negatively impacted the performance over chance for both male and female mice across sessions, with cumulative impacts of each injection. We found that animals showed reduced locomotor behavior in the chamber after saline injection. As hypothesized, acute injection stress appears to increase win-stay behavior, which may prevent animals from exploring to maximize changing reward values.

4-M-49 The Vulnerability to Social Stress in Adolescence is Sexually Dimorphic

Samuel Richer[1], Andrea Pantoja Urbán[1], Giovanni Hernandez[1], Amelie Mittermaier[1], Michel Giroux[1], Cecilia Flores[1]
[1]McGill

Social stress during adolescence increases psychiatric risk, but not all individuals are affected equally, with important sex differences. Whether short term responses to adolescent social stress modify enduring effects in males and females is unknown. Psychiatric disorders that emerge during adolescence are characterized by impulse control deficits. Here, we explored whether social approach behaviour 24h after social stress in adolescence predicts impulse control in adulthood. The adolescent social stress model used allows to test and compare male and female mice. Male and female C57BL/6J mice were exposed to a modified accelerated social defeat (AcSD) model followed by a social interaction test 24 hours later. Mice showing high social interaction were labeled as ?resilient? and socially avoidant mice were classified as ?susceptible.? In adulthood, mice were assessed in the Go/No-Go task to quantify impulse control. Compared to males(n=25), females(n=31) that underwent AcSD in adolescence showed a higher proportion of resilience to social avoidance, even when matching the number of received attacks. In adulthood, "resilient" but not "susceptible"? females showed cognitive deficits ($F(30,690)=1.949$, $p=0.0020$), compared to control counterparts. In males, both ?resilient?

and ?susceptible? groups showed impaired impulse control in adulthood. AcSD in adolescent females, but not males, led to increased body weight in adulthood ($F(134, 3082)=1.365$, $p=0.0040$), without altering motivation for food consumption. Vulnerability to the enduring effects of adolescent AcSD on cognitive function and body weight differs between male and female mice. Immediate responses to social stress in adolescence associate with adult cognitive performance in females but not males.

4-M-50 Dopamine reports reward prediction errors, but does not update policy, during inference-guided choice

Marta Blanco-Pozo[1], Thomas Akam[1], Mark Walton[1]
[1]*University of Oxford*

Dopamine is thought to carry reward prediction errors (RPEs), which update values and hence modify future behaviour. However, updating values is not always the most efficient way of adapting to change. If previously encountered situations will be revisited in future, inferring that the state of the world has changed allows prior experience to be reused when situations are reencountered. To probe dopamine's involvement in such inference-based behavioural flexibility, we measured and manipulated dopamine while mice solved a sequential decision task using state inference. Dopamine was strongly influenced by the value of states and actions, consistent with RPE signalling, using value information that respected task structure. However, though dopamine responded strongly to rewards, stimulating or inhibiting dopamine at the time of trial outcome had no effect on subsequent choice. Therefore, when inference guides choice, rewards have a dopamine-independent influence on policy through the information they carry about the world's state.

4-M-51 Nigrostriatal dopamine pathway regulates auditory discrimination behavior

Allen Chen[1], Jeffrey Malgady[1], Lu Chen[1], Kaiyo Shi[1], Eileen Cheng[1], Joshua Plotkin[1], Shaoyu Ge[1], Qiaojie Xiong[1]
[1]*Stony Brook University*

The auditory striatum, the tail portion of dorsal striatum in basal ganglia, is implicated in perceptual decision-making, transforming auditory stimuli to action outcomes. Despite its high clinical importance, the dopaminergic modulation of sensory striatal neuronal activity and its behavioral influences remain unknown. We demonstrate that optogenetic inhibition of dopaminergic projections from the substantia nigra pars compacta to the auditory striatum specifically impairs mouse choice performance but not movement in an auditory frequency discrimination task. In vivo dopamine and calcium imaging in freely behaving mice reveal that this dopaminergic projection modulates striatal tone representations, and tone-evoked striatal dopamine release inversely correlated with the evidence strength of tones. Optogenetic inhibition of D1-receptor expressing neurons and pharmacological inhibition of D1 receptors in the auditory striatum dampened choice performance accuracy. Our study uncovers a phasic mechanism within the nigrostriatal system that regulates auditory decisions by modulating ongoing auditory perception.

4-M-55 Mice with humanized Foxp2, a gene involved in language evolution, show alterations in striatal dopamine-dependent functions, striatal Foxp2 expression and in their reactivity to morphine

Christiane Schreiweis[1], Theano Irinopoulou[2], Beate Vieth[3], Lilia Laddada[4], Franck Oury[5], Eric Burguiere[1], Wolfgang Enard[3], Matthias Groszer[2]

[1]Institut du Cerveau et de la Moelle Epiniere, [2]Sorbonne Université, [3]Ludwig Maximilians University, [4]Clermont-Auvergne University, [5]Université Paris Descartes

The acquisition of language and speech is uniquely human, but how genetic changes might have adapted the nervous system to this capacity is not well understood. Two human-specific amino acid substitutions in the transcription factor FOXP2 are outstanding candidates, given its intriguing evolution and that it is the only gene firmly linked to language development. Mice carrying these two human-specific substitutions (Foxp2hum) are specifically affected in cortico-basal ganglia circuits. "humanized"Foxp2 mice proceduralize faster, a phenotype, which is associated with alterations in dopamine tissue levels and dopamine-dependent long-term depression, pointing to a tuning towards dorsolateral compared to dorsomedial cortico-striatal circuits in "humanized" Foxp2 mice. However, whether "humanizing" Foxp2 affects striatal Foxp2 expression patterns themselves in these complementary striatal subregions, had not been assessed. Therefore, we neuroanatomically characterized Foxp2 protein expression patterns in adult striatal tissue, paying close attention to striatal subregions and its compartments (striosome/matrix). Consistent with prior reports, we find that striatal neurons in "humanized" Foxp2 mice and wildtype littermates express Foxp2 in a range from low to high levels. However, we observe a shift towards more cells with higher Foxp2 expression levels in "humanized" Foxp2 mice, an effect that significantly depends on striatal region and compartment. These changes were associated with an attenuated hyperlocomotion plateau of "humanized" Foxp2 mice in a morphine sensitization assay, a treatment primarily targeting the striosome compartment.

4-M-56 A molecular map of the learning striatum

Eliana Lousada[1], Aleksandar Janjic[2], Eric Burguière[1], Wolfgang Enard[2], Christiane Schreiweis[1]
[1]Institut du Cerveau et de la Moelle Epiniere, [2]Ludwig-Maximilians University

Efficient every day skills rely on automatized behaviors. As behaviors are learnt and repeated, the link between the action and its context incrementally increases until automaticity. Key players involved in this process are cortico-basal ganglia loops (CBGs). CBGs are topographically organized into limbic, associative and sensorimotor loops coursing through the ventral, dorsomedial, and dorsolateral striatum, respectively. These circuits are recruited to different extents during learning; however, the dynamics of the region-specific molecular events have never been assessed during learning and consolidation of a skill. Aims: Detection of region-specific striatal molecular key players of skill learning and consolidation. Methods: We developed a binary visual discrimination task in fully automated operant conditioning chambers. At different learning stages (naïve, early, intermediate, and over-trained), we collected brain samples from the ventral, dorsomedial, and dorsolateral striatum of wild-type mice, and performed bulk RNA-sequencing using the SCRB-seq protocol. Results: Molecular changes of early learning distinguish themselves from changes characteristic for learning consolidation. While gene categories involved in early learning include brain plasticity and development, those of later learning stages appear more related to long-term plastic changes including long-term synaptic potentiation and dendritic spine maintenance. Throughout learning, we could also find differentially expressed genes related to dopamine signaling, and synthesis of different types of neurotransmitters. Conclusions: Striatal molecular signatures of early learning and automatization are complementary and changes in expression patterns align with expected early or long-term plastic changes.

4-M-58 Dopamine mediated effect of amphetamine on food and water intake

Miriam E Bocarsly[1], Veronica A Alvarez[1]

[1]*NIH*

Psychostimulants, such as amphetamine, which are known to increase striatal dopamine, have been shown to decrease food intake (hypophagia) and simultaneously increase water intake (polydipsia). In the current study, we used high precision food and water intake monitoring to characterize the effects of amphetamine in wildtype mice. We found that a peripheral dose of amphetamine (2 mg/kg) led to an acute decrease in standard chow intake and an increase in water intake. Further, systemic administration of quinelorane (0.03 mg/kg), a D2R-like agonist, also led to decreased chow intake and increased water intake, indicating that dopamine-mediated hypophagia and polydipsia are likely modulated by the dopamine D2 receptor (D2R). These findings are consistent with early pharmacological studies; however, little is known about the specific receptor localization nor the mechanisms by which these drugs are acting to facilitate changes in consummatory behavior. We used transgenic mouse models with targeted deletion of D2R in striatal medium spiny neurons (MSN) or dopaminergic terminals emanating from the midbrain. We found that targeted deletion of D2Rs on MSNs impairs amphetamine-induced hypophagia but has no effect on polydipsia. Conversely, mice specifically lacking D2Rs on dopaminergic terminals show impaired amphetamine-induced polydipsia, but no effect on hypophagia. Both transgenic mouse lines demonstrated intact locomotor responses to amphetamine. These data suggest that D2Rs on MSNs are required in amphetamine-induced hypophagia, while D2Rs on midbrain dopamine neurons are required in amphetamine-induced polydipsia. Better understanding of the neural circuitry underlying stimulant-induced consummatory behavior can inform the development of obesity-targeting pharmaceuticals.

N - Other

4-N-52 Role of prefrontal cortex and striatum dcc gene network in the development of cognitive control throughout childhood and adolescence

Jose Restrepo[1], Sachin Patel[1], Zihan Wang[1], Michael Meaney[1], Patricia Silveira[1], Cecilia Flores[1]
[1]McGill University

By the time humans reach adolescence, most of the neuroanatomical foundations are set in place. An important exception is the establishment of dopamine connectivity in the medial prefrontal cortex (mPFC) which reaches full maturation only in adulthood. Altered mPFC dopamine connectivity and function is associated with deficits in cognitive control in adulthood. Given the central role of the DCC gene signaling pathway in the delayed development of the mPFC dopamine circuitry, we investigated whether a polygenic score reflecting variation in the function of the DCC gene network in target regions of dopamine pathways (mPFC and striatum) is associated with measures of cognitive control in community samples of children and with psychopathology (addiction) in adults. We created a list of genes co-expressed with DCC in the mPFC and striatum, compiling single nucleotide polymorphisms from these genes in a score using the SNP-gene expression association betas described in GTeX. We created the polygenic score and investigated its ability to predict impulsive phenotypes in children, and likelihood and number of addictions in adults. A higher score was associated with higher measurements of impulsivity in 6-year old children, as measured with the Information Sampling Task (n=205) and the Stop Signal Reaction Time Task (n=398), and with a higher number of addiction comorbidities in adults (n=2719). These results provide evidence of the crucial role of the DCC gene network in the development of impulsivity in children, and of addiction vulnerability in adulthood, while also translating

observations made in rodent models where variations in levels of Dcc expression during development result in differential mPFC dopaminergic architecture and functioning later in life.

4-N-53 Sex-modulated norepinephrine function in mediating exploration-exploitation tradeoff

Cathy Chen[1], Becket Ebitz[1], Sylvia Bindas[2], Evan Knep[1], Nicola Grissom[1]
[1]University of Minnesota, [2]Oregon Health & Science University

In an uncertain world, we balance two goals: exploiting rewarding options and exploring potential better alternatives. Often, there are a range of individual solutions to this dilemma, but we know little about the neural bases of this interindividual variability. One major axis of interindividual variability in decision-making is sex. Sex is a profound modulator of the locus coeruleus-norepinephrine (LC-NE) system that has long been thought to underpin exploration. However, it remains unclear how the balance between exploration and exploitation differs across sexes, much less the role of LC-NE in these sex differences. Here, we compared male and female mice in a restless two-arm bandit task, which encourages both exploration and exploitation. The reward probabilities of two arms changed independently and stochastically over trials so that the animals could only infer values through sampling and integrating choice-outcome history. We found that that females were more likely to repeat behaviors that produced reward. Fitting reinforcement learning models revealed that the learning rate of females was higher than males and, unexpectedly, increased over time, suggesting that females "learned to learn". To determine whether increased learning in the females was due to LC-NE tone, we systemically administered propranolol, a β-adrenergic receptor antagonist. Surprisingly, this did not reduce the learning rate in females, but instead increased the stickiness of choices and reduced decision noise. Together, these results highlight sex differences in solving the explore/exploit dilemma differently and a novel role of LC-NE system in regulating exploration via regulating behavioral stickiness, rather than learning rate.

4-N-54 Novel computational models for the analysis of dopamine release kinetics in vivo from fast-scan cyclic voltammetry

Shashaank N[1], Mahalakshmi Somayaji[1], Mattia Miotto[2], Eugene Mosharov[1], David Knowles[1], Mark Wightman[3], Giancarlo Ruocco[2], David Sulzer[1]
[1]Columbia University, [2]Sapienza University, [3]University of North Carolina at Chapel Hill

Dopamine neurotransmission in the striatum is central to many normal and disease functions. Ventral midbrain dopamine neurons exhibit ongoing tonic firing that produce low extrasynaptic levels of dopamine below the detection of extrasynaptic electrochemical recordings (~10 - 20 nanomolar), with superimposed bursts that can saturate the dopamine uptake transporter and produce transient micromolar concentrations. The bursts have previously been shown to lead to presynaptic plasticity via multiple mechanisms, including the expression of the presynaptic protein α-synuclein (as well as β-synuclein and γ-synuclein), but analysis methods for these kinetic parameters are limited. To provide a deeper understanding of the mechanics of dopamine neurotransmission, we developed three computational models of dopamine release to analyze in-vivo fast-scan cyclic voltammetry recordings from the dorsal striatum of mice. The models achieve close fits to the cyclic voltammetry data and provide kinetic estimates of presynaptic facilitation and depression of dopamine levels. Consistent with prior studies in spontaneous dopamine release, we found that three kinetic components are required in the models to capture the changes in dopamine release over time: short-term facilitation, short-term

depression, and long-term depression. We used these computational models to analyze the kinetics of the synuclein family of proteins, and our results support recent findings linking α-synuclein to the short-term facilitation and long-term depression of dopamine release kinetics, as well as identify a new role for β-synuclein and/or γ-synuclein in the long-term regulation of dopamine reuptake and release.

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