ABSTRACTS

Plenary sessions

Sunday

David Attwell
Department of Neuroscience, Physiology & Pharmacology, University College London, UK

Control of cerebral and cardiac blood flow by capillary pericytes in health and disease

Brain blood flow is regulated to ensure adequate power for neuronal computation. Blood flow is increased to areas where neurons are active, and this increase underlies non-invasive brain imaging using BOLD fMRI. Blood flow is controlled at the arteriole level by smooth muscle, but there is controversy over whether it is also regulated by pericytes at the capillary level. I will demonstrate that neuronal activity mainly increases cerebral blood flow by dilating capillaries via pericytes, that this involves signalling via astrocytes, and that dilation of capillaries and dilation of arterioles are mediated by different messengers. Ischaemia leads to pericytes constricting and dying, and thus reducing blood flow, making pericytes a therapeutic target in stroke. I will show that similar events occur in cardiac ischaemia. Finally I will present preliminary data showing that pericyte-mediated constriction of capillaries may play a role in Alzheimer's disease.

Monday

Andrew Ewing[1,2], J Dunevall[2], S Majdi[1], X. Li[1], L Ren[2], D Ye[1], S Taleat[1], A Larsson[1]
[1]Department of Chemistry and Molecular Biology, University of Gothenburg, Sweden; [2]Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg, Sweden

Measuring Synaptic Vesicles Using Cellular Electrochemistry and Nanoscale Molecular Imaging

Vesicle impact electrochemical cytometry is a method whereby vesicles filled with electroactive metabolites impact an electrode surface, adsorb, and then are electroporated to expose their contents that are quantified by oxidation. Placing a nanotip electrode into a cell allows the vesicles to impact and open on this electrode tip allowing quantification of content and this is called intracellular vesicle impact electrochemical cytometry (IVIEC). Accurate determination of the contents of single nanometer neurotransmitter and hormonal vesicles is possible, and by comparison to the amount released in exocytosis, we show that about half the transmitter load of a large dense core vesicle is released during an exocytotic event, again experimentally demonstrating partial release. We have examined release and content after drugs that affect cell function and/or memory/learning (cisplatin, zinc, lidocaine, barbiturate) and find they act via changing the partial opening of the pore during exocytosis with only a few affecting vesicle content. Hence, when considering presynaptic plasticity, the dynamics and the fraction released during exocytosis events appears to be more important than changes in vesicle amount, but distribution across the vesicle are likely important to dynamics. The amine content across nanometer neuroendocrine vesicles in nerve-like cells can be imaged with NanoSIMS. Combined NanoSIMS and TEM shows the distribution profile of newly synthesized dopamine across individual vesicles. Combined NanoSIMS and IVIEC allows quantification of the mass spectrometry images across single vesicles.

Tuesday

Bita Moghaddam
Department of Behavioral Neuroscience, OHSU, Portland OR, USA

Dopamine modulation of prefrontal cortex activity is manifold and operates at multiple temporal and spatial scales

Dopamine neurotransmission in the prefrontal cortex (PFC) is critical to numerous cognitive and affective processes such as attention, working memory, action selection, behavioral inhibition, and stress response. We posit that dopamine's involvement in these temporally and functionally diverse processes requires that it influences PFC neuronal activity at multiple scales contemporaneously. To investigate this, we assessed the impact of different patterns of optogenetic stimulation of dopamine neurons on three measures of spatiotemporal spontaneous activity in the PFC: spiking of single units, population level ensemble response using a Euclidean distance state-space model, and local field potential oscillations. The most robust pattern of influence was observed after sustained phasic activation as opposed to burst activation of dopamine neurons. The former pattern of activation has been reported during tasks involving sustained attention and distant-goals whereas the latter is mostly associated with transient teaching signals. At the single unit level, a mix of excitation and inhibition was observed at transient and prolonged durations in a minority (<20%) of spontaneously active units, indicating that the direct impact of dopamine on individual neurons is heterogeneous and weak. At the ensemble level, we observed a pronounced modulation of population state-space response only during sustained phasic activation of dopamine neurons. This pattern of activation also preferentially enhanced high gamma oscillations and the coupling of gamma to theta oscillations. Thus dopamine modulation of PFC is sensitive to the pattern of dopamine neuron activation and operates by causing concomitant changes in dynamic properties of state-space interactions and gamma oscillations.

Wednesday

Ann M. Graybiel
MIT, McGovern Institute for Brain Research, Cambridge, MA, USA

Steps toward identifying functions of the striosome-matrix organization of the striatum

Within the basal ganglia, and across cortico-basal ganglia systems, increasing evidence suggests that there are powerful microcircuits with specialized functions. A potential template architecture for some of these microcircuits is afforded by the striosome-matrix organization of the striatum, wherein striatal input and output streams tend to engage either striosomes or matrisomes of the large extra-striosomal matrix. In our laboratory, we are attempting to identify the functions of striosomes, long known mainly on the basis of their neurochemical specialization and preferential input connections from limbic regions and output connections with dopamine-containing regions of the midbrain and, indirectly, the lateral habenula. I will review progress in non-human primates and rodents, pointing to a function for the striosomal system in forms of approach-avoidance decision-making that, in humans, are associated with emotional states including anxiety. These experiments have focused on the use of tasks in which subjects choose to accept or reject combined appetitive and aversive offers. These protocols allow the identification and quantification of decision boundaries. Attempts to alter these decisions through either microstimulation or optogenetics suggest that pathways leading from the medial prefrontal neocortex to the striatum, including in the primates those originating in the pregenual anterior cingulate cortex, have a strong influence on such decision-making. This work is leading to the view that striosome-matrix architecture of the striatum has functional meaning. These compartments have different neurogenetic origins, have been identified as evolutionarily ancient parts of the brain plan of vertebrates, and have in some cases been identified as being selectively vulnerable in human disorders. We hope to contribute to an understanding of how these striosomal microcircuits are integrated into forebrain networks modulating movement and emotion, in health and disease.

Symposia

Symposium 1: Marianne Fillenz Legacy Plenary Symposium

Chair: Martyn Boutelle

Martyn G Boutelle*, Michelle L Rogers, Chi Leng Leong, Isabelle Samper, Sharon Jewell
Department of Bioengineering, Imperial College London, UK

Real-time monitoring of neurochemistry - from grooming responses to human traumatic brain injury

My first contact with neurochemistry was as a tame electrochemical PDRA working within Marianne Fillenz's group. I learned neuroscience from Marianne's perspective - that while electrophysiological activity was clearly important, the key information came from how neurochemical signals interacted with (she would have said modulated) the interpretation of the electrical signals. At the start we could measure noradrenaline and dopamine synaptosomal release, and, in-vivo at 30 min intervals, ascorbic acid AA (as a correlate of glutamate) and HVA (as a dopamine metabolite) using carbon paste electrodes. With the development of new instrumentation, we measured AA and glucose amperometrically confirming that changes in both took place on fast timescale. The introduction of microdialysis (via Tyra Zetterström) greatly increased the number of neurochemicals measurable, including many neurotransmitters, at the expense of temporal resolution due to the mass sensitivity of our assays and our reluctance to change moving sample vials every 2 minutes! In response to this I launched upon building sensitive on-line microdialysis methodologies. We studied the interaction of neuronal activity with mild stimulae such as stimulated grooming, local blood flow and energy metabolism. Fast forwards to our current research we are still interested in energy metabolism, but within the context of acute human traumatic brain injury. I will described our latest wireless microfluidic devices and the need for neurochemical monitoring within an intensive care environment.

John Lowry
Neurochemistry Group, Department of Chemistry, Maynooth University, Co. Kildare, Ireland

Adventures in Electrochemistry: Monitoring Molecules in Real-Time to Understand Brain Function

I joined the Laboratory of Marianne Fillenz in the autumn of 1994 as an EC funded Marie Curie Fellow. I had developed a glucose biosensor during my PhD research work with Prof. Robert O'Neill (University College Dublin), and the goal was to use this as a complement to microdialysis studies of metabolism, which were ongoing in Marianne's laboratory at the time. It was a little daunting as a young electrochemist whose primary training was in chemistry and mathematics. However, all anxiety quickly disappeared as Marianne and her group welcomed both me and the biosensor with great enthusiasm and openness, and so began a friendship and collaboration that was active until Marianne's passing in 2012. In the early days work concentrated on measuring glucose, tissue O2 and blood flow, with the primary goal of studying the astrocyte-neuron lactate shuttle (ANLS) hypothesis. This theory, initially proposed by Magistretti and Pellerin in 1994, challenged the idea that the energy needs of neurons are met exclusively through glucose and oxidative metabolism, by proposing that L-lactate was an important alternative fuel source. This was the foundation for subsequently extending the range of sensors to include lactate, glutamate and NO, and the establishment of new collaborations (often initiated by introductions from Marianne) with scientists from different fields such as pharmacological/behavioural neuroscience and neuroimaging. My presentation will overview these interactions, highlighting their relevance to Marianne's legacy in terms of monitoring molecules and understanding brain function.

R Saylor, S Samarnayake, A Abdalla, M Hersey, **Parry Hashemi***
Department of Chemistry and Biochemistry, University of South Carolina, SC, USA

Fundamentally novel perspectives on psychiatric diseases with microengineered, electrochemical detection platforms

Dysfunctions of the brain's serotonin system are thought to underlie the symptoms of depression, primarily because antidepressants that target the serotonin transporters show some clinical success. Despite this, antidepressant efficacy is variable, often delayed and temporary. The incidences of depression are predicted to increase such that by 2013, depression is predicted to be the leading cause of disease burden world wide, thus it is imperative that antidepressant efficacy be improved. However because the roles of serotonin in the brain during health and disease are ill-defined, it

is extremely challenging for drug developers to develop better, more targeted antidepressant therapies. This situation is confounded because current therapies assume that the serotonin system dysfunctions in the same way regardless of the multitude of causes (psychological, genetic, exposure, disease) of human depression. In this work, we develop and apply novel voltammetric tools and couple them with confocal imaging, cellular biochemistry, animal behaviour and mathematical models to characterize serotonin neurotransmission in different animal models of depression. Specifically we find that alterations in another neurotransmitter system, the histamine system, likely modulate abnormalities in the serotonin system. Additionally we find that rapid changes in serotonin transporter function may be responsible for the dosing and delayed therapeutic issues of common antidepressants. We model the experimental data and offer mechanistic insights into how histamine and serotonin modulate one another in health and disease and this leads us to hypothesize why antidepressants are not universally effective. The data afforded by our chemical tools has the capacity to improve therapeutic strategies towards depression by shedding light on another important player.

Charles Marsden
University of Nottingham, UK
In discussion with Nigel Maidment and Martyn Boutelle.

Symposium 2: Location Matters: Anatomical and Functional Specialization of Dopamine Signals

Chairs: Anne Collins, Ingo Willuhn

AK Kaufmann, LA Bryden, PJ Magill, **Paul Dodson***
Medical Research Council Brain Network Dynamics Unit, Department of Pharmacology, University of Oxford, UK

Heterogeneity in the encoding of behaviour by midbrain dopamine neurons

Midbrain dopamine neurons are thought to provide a uniform teaching signal that guides learning. However, rather than being a homogeneous population, the neurons that generate this signal are not only heterogeneous in their projection targets and molecular expression profiles, but also in the way they encode different types of behaviour. To investigate how such diverse neurons generate signals that guide behaviour, we used in vivo recording and juxtacellular labelling of individual dopamine neurons in head-fixed mice. The juxtacellular technique not only permits confirmation that the recorded neuron was dopaminergic, but also determination of each neuron's precise anatomical location and expression of particular molecular markers. I will present some of our recent work, which reveals considerable diversity in the way that individual dopamine neurons encode different aspects of behaviour.

Anne Collins[1]*, 1, TJ Aitken[1], V Greenfield[1], SB Ostlund[2], KM Wassum[1,3]
[1]Department of Psychology, UCLA, USA; [2] Department of Anesthesiology and Perioperative Care, UCI, Irvine, CA, USA; [3]Brain Research Institute, UCLA, USA

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

Reward-predictive stimuli provide a major source of motivation for reward-seeking actions. Considerable evidence has implicated the nucleus accumbens core (NAc), and dopamine signaling therein, in the expression of a cue's motivational value. Interestingly, the striatal cholinergic system is capable of terminally modulating dopamine release and influencing local circuit dynamics within the NAc. This led us to hypothesize that the striatal cholinergic system may provide a regulatory mechanism over dopamine release and appetitive behaviours. However, little is known about how striatal acetylcholine regulates cue-related dopamine release to mediate cue-motivated reward seeking. Therefore, we assessed the causal role of NAc cholinergic interneuron activity in mediating the ability of a reward-paired cue to invigorate reward-seeking actions by manipulating the activity of cholinergic interneurons during a Pavlovian-to-instrumental transfer (PIT) test. We then assessed the hypothesis that activity at NAc acetylcholine receptors terminally modulates dopamine release to mediate cue-motivated reward seeking using fast-scan cyclic voltammetry. We further monitored fluctuations in NAc acetylcholine release during the PIT task using choline biosensors. Collectively, these results elucidate the role of the NAc cholinergic system in acting as a supressatory gate on cue-evoked dopamine signaling to regulate the excitatory influence of a reward-paired cue over reward seeking.

A Mohebi[1], JR Pettibone[1], AA Hamid[2], JT Wong[3], RT Kennedy[3], **Josh Berke[1]***
[1]Department of Neurology and Wheeler Center for the Neurobiology of Addiction, UCSF, CA, USA; [2] Department of Neuroscience, Brown University, Providence RI, USA; [3] Department of Chemistry, University of Michigan, Ann Arbor MI, USA

Forebrain dopamine value signals are independent of midbrain dopamine cell firing

Dopamine is critical for both reinforcement-driven learning and motivation. The role in learning is thought to involve reward prediction errors encoded by abrupt changes in midbrain dopamine cell firing. Motivational value has been proposed to be conveyed by slower, "tonic" changes in dopamine cell firing. Here we directly compared the firing of midbrain dopamine cells with forebrain dopamine release, in unrestrained rats performing an adaptive decision-making task. Using microdialysis we found that dopamine release scales with value in specific hotspots, including nucleus accumbens core and ventral prelimbic cortex. These subregions receive their dopamine input from the ventral tegmental area (VTA). However, the tonic firing of optogenetically-identified VTA dopamine cells showed no relationship to motivational value. Furthermore, during waiting periods firing of VTA dopamine cells progressively decreased, while accumbens dopamine release progressively increased, consistent with error and value coding respectively. We conclude that critical motivation-related aspects of dopamine release are controlled not by dopamine cell spiking, but instead by local influences over dopamine terminals within specific target regions.

Ingo Willuhn
The Netherlands Institute for Neuroscience, Royal Netherlands Academy of Arts and Sciences, Amsterdam, The Netherlands; Dept. of Psychiatry, Academic Medical Center, Amsterdam, The Netherlands

Regional specificity of striatal dopamine signaling during reward learning

The striatum consists of functional domains that are determined by its afferent projections, whereby the ventromedial striatum (VMS) receives predominantly limbic input and the dorsolateral striatum (DLS) mainly sensorimotor input. The release of dopamine in the striatum plays a prominent role in the regulation of these functional domains and shapes motivation, motor function, and reward learning - related to both natural and drug reinforcers. However, the exact message conveyed by dopamine signals in the striatum and its regional specificity are under active debate. Thus, we assessed dopamine release using chronically implantable microelectrodes for fast-scan cyclic voltammetry in the VMS and DLS of rats performing in different reward-driven operant conditioning paradigms. Furthermore, we investigated temporal emergence and inter-regional coordination of dopamine signals. Our results demonstrate great heterogeneity in dopamine release depending on localization in the striatum. Furthermore, we found an involvement of dopamine in both motivation and learning, different temporal emergence of regional signals during operant training, weak trial-by-trial coordination between VMS and DLS, and even a dependency of regional function on the quality of reward and/or task. Together, the presented findings demonstrate fundamentally distinct regional dopamine signaling, demanding careful future investigation of such heterogeneous dopamine release dynamics.

Julie Fudge*, Kelly EA
Del Monte Institute for Neuroscience, University of Rochester, Rochester, NY, USA

Dopamine and CRF: broadening the view

Corticotropin-releasing factor (CRF), a neuroregulator of dopamine (DA) and mediator of stress responses, is produced in cell populations throughout the brain. Cross-talk between CRF and the midbrain DA system was identified several decades ago as a mechanism by which stress can influence motivated behaviors. For example, exogenous CRF and stress both trigger DA release in the rodent 'mesocorticolimbic' path, originating in the ventral tegmental area (VTA), to precipitate relapse to addiction. Yet, the midbrain DA neurons are increasingly understood to be highly heterogeneous, with neurochemical, physiologic and connectional diversity along their mediolateral and rostrocaudal axes, which map onto specific behaviors. Thus, DA neurons within and also outside of the 'classic VTA' contribute to a variety of motivated behaviours. We recently showed in primates that extended amygdala CRF-containing afferents terminate most densely outside the 'classic' VTA, among DA neurons associated with 'limbic associative' circuits, rather than pure 'mesolimbic' paths. Moreover, many potential afferent CRF sources outside the extended amygdala are positioned to innervate other DA subpopulations, and remain largely unexplored. We will discuss a broader concept of DA circuitry, including DA subpopulation modulation by diverse CRF cell populations, can help pinpoint stressor impact on discrete DA subcircuits and behaviors.

Armin Lak*, H Gurnani, M Wells, K Harris, M Carandini
Faculty of Brain Sciences, University College London, UK

Projection-specific roles of dopamine neurons in decision making

Recent studies suggest that subpopulations of dopamine (DA) neurons with different projection targets play different roles in reward and action processing. To test the roles of these circuits in choice behaviour, we trained mice in a decision task and characterized the effects of optogenetic activation of DA neurons or their projection terminals on mice choices. In each trial, the headfixed mouse indicated the position of a stimulus appeared on the monitor by turning a steering wheel. We paired the water reward that followed one choice option with optogenetic DA stimulation. Following stimulation of VTA DA cell bodies or their terminals in ventral striatum, mice developed a tendency towards choices paired with such stimulations. This tendency affected the psychometric bias parameter, consistent with increased value of the stimulated choice option. In contrast, following stimulation of dorsal striatal DA terminals mice tended to take one action direction depending on the stimulated hemisphere. This tendency changed the psychometric lapse parameter indicating that dorsal striatal DA terminals are involved in response execution and not value processing. We propose a novel normative computational model that accounts for our observations. Together, these results illustrate distinct roles of subpopulations of DA neurons in decision making under uncertainty.

Symposium 3: New Advances in Monitoring the Release and Function of Neuropeptides in the Brain

Chairs: Leslie Sombers, Paul Slesinger

E Lacin[1], E Aisenberg[1], C Foo[2], A Lozada[2], D Kleinfeld[2], **Paul Slesinger***
[1]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY USA; [2]Department of Physics, UCSD, CA, USA

Development of optical sensors for detecting neuropeptide release in vivo

Neuropeptides are essential neuromodulators in the brain that diffuse over long distances and signal through G protein coupled neuropeptide receptors. Pharmacological and genetic studies have implicated neuropeptide signaling in brain dysfunctions, such as migraines, addiction, motivation and stress, but their precise role is poorly understood. One obstacle to elucidating their function has been a paucity of tools for detecting when and where neuropeptides are released in vivo. Here we describe an optical biosensor that can be implanted in vivo for detecting the release of a neuropeptide. Based on the design of cell-based neurotransmitter fluorescent engineered reporters (CNiFERs), we report on the creation and characterization of three new CNiFERs for detecting the neuropeptides somatostatin (SST), vasoactive intestinal peptide (VIP) and orexin.

Leslie Sombers*, CA Lee, SE Calhoun, CJ Meunier, KE Butler, GS McCarty
Department of Chemistry, Keck Center for Behavioral Biology, NC State University, USA

Chasing the Enkephalins - Electrochemical Measurements of Real¬-Time Opioid Peptide Fluctuations in the Midbrain and Striatum

The role of mesolimbic opioid peptides in motivated behavior and reward-related decision-making is unclear, despite extensive evidence indicating that these molecules are important mediators of hedonic and motivational aspects of reward processing, and the fundamental response to drugs of abuse. This is largely due to a critical gap in understanding when and where these molecules are released. We have established the feasibility of using fast-scan cyclic voltammetry (FSCV) and carbon-fiber microelectrodes for the detection of endogenous enkephalin (ENK) fluctuations in real time. By combining multiple scan rate voltammetry with constant-potential amperometry in every voltammetric sweep, putative M-ENK fluctuations and striatal dopamine (DA) dynamics are recorded at both nodes of the mesolimbic dopamine (DA) system in response to treatments that elicit robust DA fluctuations in intact striatal tissue (cocaine, L-DOPA, and unexpected food reward). The data suggest that endogenous opioid peptides may modulate DA release in these regions; however, further experiments are needed. Importantly, these measurements can serve to clarify the modulatory role of opioid peptides in a broad spectrum of pathological disorders related to dysfunction of the DA system.

Elyssa Margolis
Department of Neurology, The Wheeler Center for the Neurobiology of Addiction, UCSF, USA

Striking differences in the neuronal actions of endogenous opioid peptides with similar binding profiles

The opioid receptor family of GPCRs generally couple to inhibitory signalling pathways. Although synthetic agonists show selectivity between opioid receptors, several endogenous peptide agonists such as met-enkephalin, leu-enkephalin, and b-endorphin have similar affinities for mu and delta opioid receptors (MORs and DORs). Additionally, dynorphin-A, usually classified as a kappa opioid receptor (KOR) agonist, may also bind to MOR or DOR. I have recently shown that most ventral tegmental (VTA) neurons express both MOR and DOR. However, the actions of the MOR/DOR binding peptides in vivo, including which receptors contribute, have not been systematically compared. Using whole cell recordings in VTA neurons we found that some neurons responded similarly to met-enkephalin, leu-enkephalin, and b-endorphin. Surprisingly, approximately half of the neurons showed dissimilar responses, including opposing effects of met-enkephalin and leu-enkephalin. Further, concomitant administration of MOR and KOR selective antagonists was often required to completely block dynorphin-A responses. Therefore, despite similar binding profiles, endogenous opioid peptides can elicit strikingly different downstream signalling, even in the same neuron. Thus, to fully understand endogenous opioid function, it is necessary to determine the species of peptide released, the receptors expressed in the target tissue, and the signalling produced in the neurons of interest.

I Gomes, E Margolis, **Lakshmi Devi***
Department of Pharmacological Sciences, Icahn Medical School at Mount Sinai, New York, NY, USA

Exploring the Mysteries of the Endogenous Opioid System

The three types of opioid receptors (μ, δ, or κ) are activated by a number of endogenous opioid peptides. These peptides exert their biological effects primarily through activation of the Gαi-mediated signalling. Recent studies have begun to explore biased signalling by opioid receptors and have examined their ability to activate the G protein-independent, β arrestin-mediated signalling. In this study we have systematically examined the Gαi protein-mediated and β arrestin-mediated signalling by a panel of 22 endogenous opioid peptides at δ, μ, or κ opioid receptors. The results show that, whereas many of the endogenous peptides display a signalling pattern similar to that of classic ligands, some show interesting differences. Endorphins, classically thought to activate μ receptors, activate δ receptors with higher potency in the β arrestin recruitment assay. Similarly dynorphins, classically thought to activate κ receptors, activate all three receptors when examined for β-arrestin recruitment. These results indicate that endogenous opioid peptides exhibit biased signalling and this is likely to modulate the spatio-temporal dynamics of receptor signalling where the receptors are co-localized and/or the peptides are co-released. Studies exploring the activity of receptors in vivo have begun to support such a possibility.

Gianluigi Tanda*, MCH Rohn[3], AH Newman[1], MA Coggiano[1], C Zanettini[1], JD keighron[1], JL Katz[2], L Leggio[3], MR Lee[3]
Medication Development Program, NIDA-IRP, USA [1]; Psychobiology Section, NIDA-IRP, USA[2]; Clinical Psychoneuroendocrinology and Neuropsychopharmacology Section NIAAA, NIH/DHHS, USA[3]

Systemic oxytocin affects the reinforcing and neurochemical effects of methylphenidate

The hypothalamic neurohormone oxytocin and the neurotransmitter dopamine play a significant role in social bonding. Dopamine is also involved in both natural and pathologic behaviours related to reward and reinforcement, including substance use disorders. Early reports have shown that oxytocin might interfere with psychostimulant behavioural effects. Here, the effects of oxytocin were assessed in Sprague-Dawley rats: a) trained to intravenously self-administer methylphenidate (1 mg/kg, fixed ratio 5) during single daily 1-hr sessions, b) implanted with microdialysis probes to assess methylphenidate (0.1-1 mg/kg, i.v.) effects on levels of dopamine in the nucleus accumbens shell and core, and c) placed in infrared activity monitors to measure behavioural activation.

Oxytocin (0.1-2mg/kg, i.p., 10 min before the sessions) dose-dependently decreased maximal self-administration of methylphenidate (0.03-1.0 mg/kg/injection, i.v.). Oxytocin (0.2-2 mg/kg, i.p.) had little to no significant effects on extracellular levels of dopamine in the accumbens shell, but it dose-dependently enhanced methylphenidate-stimulated (0.1-1 mg/kg, i.v.) dopamine levels in the shell (but not in the core) of the accumbens. Similar effects on methylphenidate neurochemistry were obtained when oxytocin was locally infused into the accumbens shell by reverse

dialysis, suggesting these actions being mediated by its receptors in this area. Interestingly, enhancement of dopamine levels did not result in stimulation of behavioural activities at levels higher than those produced by methylphenidate alone. Voltammetry studies are ongoing to evaluate if oxytocin effects on methylphenidate are the results of changes in dopamine uptake or release. The present results suggest that oxytocin attenuates the psychostimulant-like reinforcing effects of methylphenidate, likely acting through altered dopamine neurotransmission in the terminals of the mesolimbic system. Thus, these results confirm and extend the potential translational therapeutic value of oxytocin for psychostimulant use disorders.

Research Funded by: MDP-NIDA-IRP, NIH/DHHS; Bench-to-Bedside Grant by the OBSSR/NIH; Intramural Grant ZIA AA000218-01 (CPN Section) DICBR/NIAAA/NIH/DHHS

Xiuying Li[1+], Emre Lacin[2+], David Kleinfeld[3], Paul A. Slesinger[2]#, **Zhenpeng Qin[1]#***

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[+]: co-first authors; #: co-corresponding authors.

Two-photon uncaging of neuropeptides

Neuropeptides, which function in parallel with classical neurotransmitters, are implicated in cognition, sensorimotor processing and controlling blood flow. Although widely expressed in the brain, studying the effect of endogenously released neuropeptides in vivo has been hampered by inadequate techniques for controlling the release of neuropeptides. Here, we describe the development of a new optical tool for releasing neuropeptides with temporal and spatial precision. Specifically, we are developing two-photon (2p) uncaging of neuropeptides that are packaged inside nano vesicles, constructed from phospholipid liposomes coated with gold nanoparticles. Our preliminary data demonstrate in vitro uncaging of somatostatin (SST), which was monitored by a new cell-based neurotransmitter fluorescent engineered reporter (CNiFER) for SST. Furthermore, we have tested the 2p uncaging of a fluorescent dye (620 daltons) in vivo using nano-vesicles. Work is ongoing to investigate the in vivo uncaging and monitoring of SST and other neuropeptides. Controlling the release of neuropeptides in real-time in awake animals performing complex behaviors would be transformative, enabling the elucidation of the function of neuropeptides in regulating neural circuits in the brain.

Symposium 4: Gaining Novel Molecular Insights into CNS Disease Processes with Electrochemical Sensors and Biosensors

Chairs: Gary Gilmour, John Lowry

Ilse Smolders*, Y Van Den Herreweghen, L Walrave, J Bongaerts, Y Van Wanseele, A Van Eeckhaut, D De Bundel

Department of Pharmaceutical Sciences (FASC laboratory), Research Group Experimental Pharmacology, Center for Neurosciences (C4N), Brussels, Belgium

Chemogenetic modulation of specific brain cell types for monitoring gliotransmitter and neuropeptide release

Chemogenetics is based on virus-driven expression of genetically modified receptors in a cell type of interest and selective activation of these receptors by an otherwise biologically inert ligand, the so-called 'Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)' approach. This technique is now adopted in our laboratory aiming to modulate astrocytes and neuromedin U-containing neurons. Preliminary data will be shown; encountered difficulties and future challenges will be discussed. Technical innovation includes the possibility to monitor in vivo gliotransmitter release from astrocytes as well as in vivo neuromedin U release upon chemogenetic activation. We finally aim to answer fundamental questions regarding the contribution of astrocytes and neuromedin U-containing neurons to epileptic seizure generation and stress-induced psychopathology respectively.

Irina Ionescu, **Kelly Allers***
Boehringer Ingelheim Pharma GmbH &Co. KG, CNS Diseases Research, Germany

Glutamate Biosensors: an Industry Experience

At Boehringer Ingelheim we strive to approach CNS Diseases Research with an RDoc focus, linking specific brain circuits to symptoms. As part of this approach, we have recently established a glutamate biosensor group, with the aim of using this technology to probe glutamate circuits. One specific focus in our lab is the glutamate response to stress in different brain regions, and for this we incorporate both acute stress with a 5-minutes restraint stress, and chronic stress in the form of chronic corticosterone treatment. Acute restraint stress induces an increase in glutamate in both the prefrontal cortex and the amygdala in the range of 200-300nM. Chronic corticosterone treatment potentiates the stress response. We are continuing to develop this model further, with the intention of finding the mechanisms involved in this hyper-stress response as part of our search for new targets to treat stress-related diseases. A second focus in the lab is validating the mechanism of action of known molecules. For example, we have recently demonstrated that clinically effective fast-acting NMDA antagonists produce a consistent and dose dependent increase in glutamate levels in the medial prefrontal cortex, and this effect can be separated from psychotomimetic actions.

LM Teles-Grilo Ruivo, KL Baker, MW Conway, PJ Kinsley, G Gilmour, KG Phillips, JTR Isaac, JP Lowry, **Jack Mellor***
Centre for Synaptic Plasticity, School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK; Lilly Centre for Cognitive Neuroscience, Eli Lilly and Company Ltd., Surrey, UK; Department of Chemistry, Maynooth University, Co. Kildare, Ireland

Coordinated acetylcholine release in prefrontal cortex and hippocampus measured by choline biosensors is associated with arousal and reward on distinct timescales

Cholinergic neurotransmission throughout the neocortex and hippocampus regulates arousal, learning and attention. However, owing to the poorly characterized timing and location of acetylcholine release, its detailed behavioral functions remain unclear. Using electrochemical biosensors chronically implanted in mice we made continuous measurements of the spatiotemporal dynamics of acetylcholine release across multiple behavioral states. We found tonic levels of acetylcholine release were coordinated between the prefrontal cortex and hippocampus and maximal during training on a rewarded working memory task. Tonic release also increased during REM sleep but was contingent on subsequent wakefulness. In contrast, coordinated phasic acetylcholine release occurred only during the memory task and was strongly localized to reward delivery areas without being contingent on trial outcome. These results show that coordinated acetylcholine release between the prefrontal cortex and hippocampus is associated with reward and arousal on distinct timescales, providing dual mechanisms to support learned behavior acquisition during cognitive task performance.

Jennifer Li
Translational & Integrative Neuroscience, Eli Lilly and Co., Surrey, UK

The utility of functional connectivity measures in AD mouse models

Alzheimer's disease (AD) treatment strategies have typically focussed on disease modification, attempting to halt or reverse characteristic progressive pathological changes in amyloid or tau proteins. However, recent neuroimaging studies suggest that there may be a prolonged phase of regional and/or neuronal network dysfunction prior to the onset of more overt pathology and symptoms. Resting state fMRI (rsfMRI) measures of functional connectivity propose a progressive decline in network connectivity related to disease severity, possibly preceded by a compensatory hyperconnectivity phenomenon at the earliest stages of disease.

While there are a great variety of AD transgenic mouse models, little work has been done so far to determine whether any of them also exhibit altered properties of their functional connectome. Such hypotheses can now be tested in awake behaving animals using in vivo oxygen amperometry as a preclinical surrogate for the human BOLD fMRI signal.

This talk reviews translational correspondences between rodent oxygen amperometry and human BOLD rsfMRI studies, firstly discussing the recent demonstration of task-induced modulation of rodent default mode network connectivity. Connectivity changes in tau AD mouse models will also be considered, hopefully demonstrating how such work may ultimately lead to earlier tracking of AD progression and facilitation of therapeutic response.

Michael Johnson*, KJ Garcia, T Schneider, MJ Sofis, SM Lemley, SV Kaplan and DP Jarmolowicz
Department of Chemistry, University of Kansas, Lawrence, KS, USA; Department of Applied Behavioral Science, University of Kansas, Lawrence, KS, USA

Mechanisms of chemotherapy-induced impairments in executive function

Chemotherapy-related cognitive impairment (CTRC, 'chemobrain'), an impairment of executive function caused by administration of adjuvant chemotherapy for non-central nervous system malignancies, is expected to affect one third of the 19 million cancers survivors in the next decade. Although recent studies have implicated a variety of causes, such as the formation of reactive oxygen species and inflammation, the specific mechanisms that underlie cognitive impairment are still not clearly defined. Our group has found previously that dopamine and serotonin release are impaired in rats treated with carboplatin. In this work, we will report on neurochemical methods combined with enhanced behavioral methods designed to identify how specific components of executive function are affected. Moreover, we have previously treated rats with KU-32, a heat-shock protein inhibitor, and found that this drug prevents the onset of cognitive impairment in rats. We will discuss potential neurochemical and molecular mechanisms that underlie this beneficial effect.

Symposium 5: Neurobiology of Nutrient Selection

Chairs: Jaime McCutcheon, Barbara Ferry

Denis Burdakov
The Francis Crick Institute, 1 Midland Road, King's Cross, London

Hypothalamic control of action-selection

The lateral hypothalamus is a classic "hunger and arousal" centre. It is now known to contain several molecularly-distinct, brain-wide projecting neural classes, such as orexin/hypocretin neurons, which control brain state and behaviour by co-releasing fast-acting amino-acid transmitters and neuropeptides. The talk will focus on our recent experiments dissecting the roles of defined classes of lateral hypothalamic neurons in different fundamental behaviours, using optogenetic circuit mapping, in vivo calcium imaging during behaviour, chemogenetic circuit interference, and classic pharmacological and behavioural approaches.

Samantha Fortin*, MF Roitman
University of Illinois at Chicago, Chicago, IL,USA

Physiological need gates taste-selective phasic dopamine responses in the nucleus accumbens

Maintaining homeostasis requires behaviours directed towards the selection and consumption of specific nutrients in times of physiological need. The neurobiological substrates that direct motivated behaviours at physiologically-relevant stimuli must therefore be in tune to both physiological state and identifying properties of the stimulus (i.e. taste). While nucleus accumbens phasic dopamine signalling is likely involved in generating nutrient-directed motivated behaviours, the state and taste-dependency of phasic dopamine signalling is underexplored. Here, we use fast-scan cyclic voltammetry to record phasic dopamine responses in the nucleus accumbens to intraoral infusions of sodium chloride and water in homeostatically balanced, sodium deplete, and water deprived conditions. Increases in dopamine concentration are observed only under conditions of physiological deficit. Furthermore, the dopamine increases are taste selective and limited to those which satisfy the need state of the animal. Thus, dopamine neurons track fluid balance and respond to sodium and water stimuli in a state and taste-dependent manner. Using immunohistochemistry and tracing techniques, we identify two sodium deprivation-responsive projections from the pons to the VTA that potentially increase the excitability of VTA dopamine neurons under sodium deplete conditions. These projections represent components of a circuit that may drive state-specific dopamine signalling and underlying motivated behaviour.

S. Guo, **Stephanie Borgland***
Hotchkiss Brain Institute, University of Calgary, Canada

Acute fasting alters dopamine release in a region and sex-dependent manner

Dopamine in the dorsal and ventral striatum is important for energizing goal directed behaviour for food. Chronic food restriction decreases terminal dopamine levels, and re-feeding enhances dopamine release. However, less is known about how acute fasting, commonly associated with 'dieting', influences dopamine release in efferent targets. We hypothesized that acute fasting alters dopamine release and reuptake kinetics in the dorsal and ventral striatum. We used fast-scan cycle voltammetry to electrochemically detect evoked dopamine concentration in the ventral striatum (nucleus accumbens core and shell) and dorsal striatum (dorsolateral and dorsomedial) of control or acutely fasted (16h) male and female C57BL6 mice. Fasted female mice had decreased evoked dopamine in the dorsal medial striatum under phasic-like firing conditions. Consistent with an increase in presynaptic inhibition of dopamine release, fasted female animals had increased responses to D2 receptor stimulation. In contrast, male mice had increased evoked dopamine in the dorsal medial striatum. However, D2 receptor responses were not different between fasted and unfasted male mice. Taken together, these results suggest acute fasting differentially alters evoked dopamine in male and female mice in a regionally selective manner. Fasting-induced decreases in striatal dopamine release may conserve resources during energy depletion.

KM Nesbitt[1], D Sandoval[2], RT Kennedy[3], **Carrie Ferrario[3]***
[1]Department of Chemistry, Towson University, USA; [2] Department of Surgery University of Michigan, USA; [3]Department Pharmacology, University of Michigan, USA

Oral and gastric sucrose produce alterations in striatal glucose, glutamate, glutamine, and GABA in obesity-prone vs. obesity-resistant rats; implications for obesity

Obesity is a rapidly growing epidemic effecting ~30% of the global population. Studies in humans suggest that inherent differences in striatal responses to food may drive over-consumption. Selectively bred obesity-prone (OP) and obesity-resistant (OR) rats are a useful model for examining basal differences, and show differences in striatal excitability and cue-triggered motivation, consistent with human data. However, nothing is known about neurochemical responses to the ingestion of food in this model. Here, we used in vivo microdialysis coupled with LC/MS/MS, to examine the neurochemical response to sucrose in the nucleus accumbens (NAc) of OP and OR rats. Rats were given sucrose pellets or intra-gastric sucrose infusion. Ingestion of sucrose pellets caused a greater increase from baseline in NAc glucose, glutamate, glutamine, and GABA in OR vs OP rats. Similarly, gastric infusion of sucrose produced an increase in NAc glucose that was greater in ORs. To differentiate changes in exogenous vs endogenous glucose, 13C6-glucose was infused intra-gastrically or given orally. Initial results suggest that changes in brain glucose are due to both local glucose production and entry of peripheral glucose. These data reveal differences in the rate and magnitude of neurochemical responses to sucrose ingestion in OP vs OR rats.

Anna Thinnes*, J Klein
Department of Pharmacology, Goethe University Frankfurt, Germany

Food-induced changes of acetylcholine in mouse hypothalamus

Feeding behaviour is controlled in the hypothalamus by humoral signals and afferent neuronal pathways including central cholinergic pathways. For instance, anorexigenic POMC (proopiomelanocortin) neurons receive cholinergic input. Here, we used microdialysis in wild type mice (C57Bl/6JRj) to monitor cholinergic activity in the hypothalamus. Food intake after an overnight fast increased extracellular ACh twofold in the hypothalamus. The effect lasted for about 60 minutes. Food containing no calories (kaolin pellets), or food that was presented but not accessible for the mice also increased ACh release. In contrast, injections of glucose or ß-hydroxybutyrate did not change extracellular ACh. We conclude that the increase of ACh in the hypothalamus was not caused by local detection of nutrients but by expectation of food intake, possibly involving motivational circuits in the basal forebrain.

Symposium 6: Explorations of the Molecular Basis of Psychiatric Illnesses

Chair: Liz Tunbridge

Marios Panayi#[1]*, Thomas Jahans-Price#[1]*, T Boerner#[1], A Huber[1,2], ME Walton[1], DM Bannerman[1]
#Joint first author
Dept. Psychiatry[1] and Dept. Experimental Psychology[2], University of Oxford, UK

Glutamatergic dysfunction leads to a hyper-dopaminergic phenotype: Linking dopamine to aberrant salience

Aberrant salience, the inappropriately persistent and high levels of attention paid to stimuli, is the dominant mechanism of psychosis in schizophrenia and thought to be mediated by elevated levels of dopamine. Recent large scale GWAS meta-analyses have established a significant association between schizophrenia and the Gria1 locus coding for the GluA1 subunit of the AMPA glutamate receptor. GluA1-KO mice have previously been studied in relation to schizophrenia but, notably, striatal whole tissue levels of dopamine and its metabolites appear normal in these animals. However, it has yet to be determined whether they exhibit dynamic, behaviour-dependent and stimulus-specific changes in dopamine.

To test this we recorded dopamine signals in the nucleus accumbens using fast-scan cyclic voltammetry in behaving wild-type and GluA1-KO mice. Neutral light stimuli evoked prominent dopamine signals in all mice. Crucially, these failed to habituate in GluA1-KO mice, resulting in a behaviourally-relevant, hyper-dopaminergic phenotype in these animals. In addition, dopamine responses to unsignalled rewards were also significantly enhanced in the knockout mice. However, preliminary data suggests that evoked dopamine in anaesthetised GluA1-KO mice is no different to WTs. Thus, we provide evidence for behaviourally-relevant hyper-dopaminergic responses in a genetically modified mouse model of glutamatergic dysfunction relevant to schizophrenia.

Jeff Dalley
Department of Psychology, University of Cambridge, UK

Distinct contributions of cortical and subcortical molecules to behavioural impulsivity: beyond the usual suspects

Behaviour expressed prematurely without foresight defines an important aspect of impulsivity. Although such behaviour can speed decision-making it often leads to undesirable consequences, including the emergence of addiction in some individuals. Since drugs that boost catecholamine transmission (e.g. methylphenidate, atomoxetine) have been the mainstay of treatments to reduce impulsivity, research in this area has unsurprisingly focussed on the central noradrenergic and dopaminergic pathways. However, recently, we have discovered that naturally-occurring impulsivity in rats, defined by a greatly increased tendency to respond before the onset of a reward-predictive stimulus, is predicted by abnormalities in GABA and myoinositol signalling in the ventral striatum and prefrontal cortex (PFC), respectively. Specifically, we found using high resolution magnetic resonance imaging and spectroscopy that high-impulsive rats exhibit diminished GM density, GABA content and markers of dendritic spines in the nucleus accumbens (NAcb), with accompanying reductions in levels of myoinositol in the PFC. Using antisense and other gene silencing approaches we determined that the high-impulsive phenotype could be reproduced by locally knocking down enzymes responsible for the formation of GABA in the NAcb (glutamic acid decarboxylase) and myoinositol in the PFC (inositol monophosphatase). The relevance of these findings for impulse control disorders in humans will be discussed.

Oliver Howes*, D Bonsall, S Jauhar, M Kokkinou, R McCutcheon, M Nour
MRC London Institute of Medical Sciences (Imperial College) and King's College London, UK

The role of dopamine and glutamate in psychotic disorders: multi-modal clinical and preclinical imaging findings

Background: A prevailing hypothesis is that glutamatergic dysregulation due to NMDA receptor hypofunction leads to disinhibition of subcortical dopaminergic function, and this alters the processing of information to lead to psychosis. We sought to investigate the relationship between dopamine and glutamate in a series of clinical imaging and preclinical chemoPET studies.

Methods: [18F]-DOPA PET was used to index dopamine synthesis capacity in the striatum and [1H]-MRS was used to index glutamate levels in the anterior cingulate cortex in people in the prodrome and with first episode psychosis (n=35), and response to antipsychotic treatment was determined. Mice received [18F]-DOPA PET following sub-chronic treatment with the NMDA blocker ketamine to determine the effect of NMDA blockade on striatal dopamine synthesis.

Results: People in the prodrome to psychosis showed elevated striatal dopamine synthesis (d=0.8, p<0.001), which was directly associated with symptom severity and increased with the transition to psychosis. Patients with first episode psychosis also showed elevated striatal dopamine synthesis capacity and this was directly related to subsequent response to treatment. Greater striatal dopamine synthesis capacity was associated with lower cortical glutamate levels. Mice treated with ketamine showed a persistent increase in dopamine synthesis capacity.
Conclusion: These findings are consistent with the hypothesis that subcortical dopamine dysfunction underlies the development of psychosis, and that this is secondary to cortical glutamatergic dysfunction. Moreover they identify the regulation of dopamine synthesis as a target for new treatments for psychosis.

Simon Lovestone
Department of Psychiatry, University of Oxford, UK

Blood protein biomarkers and therapeutics for Alzheimer's disease
Blood based protein markers for Alzheimer's disease have been sought as a less invasive alternative to PET or CSF markers now available to estimate Amyloid and Tau pathology. Case control proteomic studies have shown a signal in blood but have suffered from only modest replicability across studies and unstable technical platforms. Adopting an alternative approach, predicated not on disease category but on measures of pathological load, we have used mass spectrometry, immunocapture and aptamer capture to identify a panel of proteins that replicate across multiple studies and might be used to help reduce the cost of screen failure and time to participant entry in experimental studies of disease modification therapies in AD. Together with companion markers for specific therapies, such approaches amount to a form of precision medicine that promises to substantially enhance neurodegeneration disease research.

Lauren Burgeno[1,2,3]*, NL Murray[1], RD Farero[1], JS Steger[1,2], ME Soden[1,2], I Willuhn[1], LS Zweifel[1,2], PEM Phillips[1,2]
[1]Department of Psychiatry & Behavioral Sciences, University of Washington, USA; [2]Department of Pharmacology, University of Washington, USA; [3]Current Address: Department of Experimental Psychology, University of Oxford, UK

Diametric Changes in Striatal Dopamine Release Underlie Drug-Taking and Drug-Seeking Behaviors
Though altered dopamine transmission is implicated in most contemporary theories of addiction, the timing, context, and directionality of these changes remain a matter of debate. While some studies demonstrate dopamine in the nucleus accumbens core (NAcc) plays an important role in producing drug satiety, others suggest NAcc dopamine mediates craving and promotes drug seeking. How might drug-cue elicited dopamine transmission in the NAcc serve both as a satiety signal and to produce 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, presented unexpectedly during abstinence, signal possible drug availability nearby and promote drug-seeking. For NAcc dopamine to both decrease drug-taking and increase drug-seeking, we hypothesize there must be a divergence in dopamine evoked by drug-paired cues when presented in drug-taking vs. -seeking contexts. To test this hypothesis, we used fast-scan cyclic voltammetry to measure changes in drug-cue elicited dopamine over time in both of these contexts. Indeed, we find that while cue-elicited dopamine transmission significantly decreases during drug-taking ($p<0.05$, as we previously published), dopamine responses to the same cue increase during drug-seeking ($p<0.01$).

Erik Carlson[1]*, SG Sandberg[1], TM Locke[1], PEM Phillips[1,2], and LS Zweifel[1,2]
[1]University of Washington, Department of Psychiatry and Behavioral Sciences; [2]University of Washington, Department of Pharmacology

Genetic Dissection of Catecholaminergic Innervation of the Cognitive Cerebellum
Studies in humans and non-human primates have identified a region of the dentate nucleus of the cerebellum (DCN), or lateral nucleus in rodents (LCN), which is activated during performance of cognitive tasks and is implicated in psychiatric illnesses. We have shown that the dopamine D1 receptor marks a population of LCN neurons with similar spatial distribution and regulates performance on tasks related to navigation and working memory, and connects with other parts of the brain classically involved in these functions. However, virtually nothing is known about the basic anatomical and functional organization of the LCN. We hypothesized that the locus coeruleus (LC) is the principal source of catecholamine release in LCN, and that catecholamines are required for cerebellar enhancement of cognitive tasks.

Mapping experiments in mice revealed projections of the LC to DCN, and stimulation of LC resulted in catecholamine release in the LCN measured with fast scan cyclic voltammetry. When tyrosine hydroxylase expression is genetically inactivated in input projections to the LCN, abnormal performance on discrimination of fear predictive cues, working memory, and impulsive behaviors is observed. Finally, we have preliminary data showing fluctuation of catecholaminergic release in voltammetric recordings from LCN in freely moving mice.

Symposium 7: Exploring Extracellular Space on Different Spatial Scales

Chairs: Charles Nicholson, Sabina Hrabetova

Sabina Hrabetova
Department of Cell Biology, State University of New York Downstate Medical Center, USA

Exploring the structure of brain extracellular space with diffusion analysis using Real Time Iontophoresis

Brain extracellular space (ECS) is a system of interconnected pores filled with ionic solution and macromolecules of extracellular matrix. ECS channels chemical signals between cells and is an essential route for delivery of nutrients and drugs. Diffusion experiments employing the Real Time Iontophoresis (RTI) method quantify two macroscopic structural parameters of the ECS: volume fraction, which is the proportion of tissue occupied by the ECS, and diffusion permeability (inversely related to the square of the tortuosity), representing delays imposed on the diffusing molecules by obstacles in the tissue.

Over past 35 years, studies utilizing the RTI method have shown that the ECS occupies about 20% of brain tissue and that diffusion permeability for small molecules is about 0.39. Recent work suggests that ECS volume is not static but that it fluctuates during physiological brain states, such as the sleep-wake cycle, and during pathological conditions, such as epileptic seizures.

Complex ECS geometries and their dynamic nature require that the RTI method is combined with numerical models that aid data interpretation. This is the case of lower diffusion permeability arising from dead-space microdomains or interpretation of diffusion in layered regions such as hippocampus.

Charles Nicholson
Department of Neuroscience & Physiology, New York University School of Medicine, USA

Integrative Optical Imaging of macromolecular diffusion reveals origins of structural hindrance in extracellular microenvironment

Integrative Optical Imaging (Biophys. J. 65:2277, 1993) employs a micropipette point-source to release macromolecules labelled with a fluorescent dye into superficial brain tissue or a brain slice. The diffusing molecules are imaged with an epifluorescent microscope and recorded with a digital camera. This projects the 3D cloud of molecules onto the 2D image plane of the camera and by considering the optical point-spread function it may be shown that the intensity distribution is Gaussian, enabling an effective diffusion coefficient and tortuosity to be extracted.

Experiments using dextran molecules ranging from 3 – 525 kDa, and proteins, including albumins, epidermal growth factor and IgG, as well as a pegylated quantum dot have shown that tortuosity increases with molecular size and the relationship may be fitted by restricted diffusion theory. This theory assumes that large molecules experience steric hindrance and interact with the walls of the pore space. If the pore geometry is represented by planar sheets, this theory predicts a 'typical' width of about 40 nm (http://dx.doi.org/10.1016/j.bpj.2017.06.052). The interpretation of these results has been explored in model geometries that also included dead-space microdomains using the Monte Carlo simulation software MCell (www.mcell.org).

Kaiyu Zheng[1]*, TP Jensen[1], LP Savtchenko[1], JA Levitt[2], K Suhling[2], **Dmitri A. Rusakov[1]***
[1] Institute of Neurology, University College London, UK; [2] Department of Physics, King's College London, UK

Measuring nanoscale diffusion in the synaptic cleft and beyond with time-resolved fluorescence anisotropy

Brain activity relies on rapid molecular diffusion within nanoscopic spaces occurring outside and inside nerve cells, such as synaptic clefts or dendritic spines. Measuring diffusion on this small scale in live brain tissue has not hitherto been possible. However, this knowledge is important for understanding the dynamics of molecular events and electric currents that shape physiological signals throughout neural circuits. We have advanced time-resolved fluorescence anisotropy imaging combined with two-photon excitation microscopy to map nanoscale diffusivity in ex vivo brain slices. We find that in the brain extracellular space small water-soluble molecules move on average ~30% slower than in a free medium whereas inside neuronal dendrites this diffusion retardation could be as high as ~70%. We find that inside the synaptic cleft of the connections formed between hippocampal granule cells and CA3 pyramidal cells nanodiffusion is decelerated by ~46%. These quantities provide previously unattainable basic constrains for the receptor actions of released neurotransmitters. They should also guide our estimates of the electrical conductance in the brain interstitial medium and the limiting rate of molecular interactions or conformational changes in the microenvironment of central synapses.

Juan Varela[1]*, A Godin[2], Z Gao[2], L Cognet[2], L. Groc[1]
[1]Interdisciplinary Institute for Neuroscience, CNRS and University of Bordeaux, France; [2]LP2N, CNRS, Institut d'Optique and University of Bordeaux, France

Super-resolving the nanoscale organisation of the extracellular space of the brain tracking carbon nanotubes

The brain is a dynamic structure where its extracellular space (ECS) occupies almost a quarter of its volume. The properties of this ECS are highly regulated and are key for signalling, synaptic transmission, nutrient distribution and clearance of toxic metabolites among others. The spatial complexity of this space is such that conventional biophysical techniques can't reveal its structure unless the tissue is fixed. In order to study the ECS in living tissue we developed a novel delocalized and non-inflammatory way of delivering nanoparticles in vivo to the brain. This allowed us to study single-nanoparticle diffusion in living tissue. By tracking single-walled carbon nanotubes in we directly studied the local ECS structure and rheology. Imaging at wavelengths in the near-infrared spectrum we could access several tens of microns inside the living tissue and obtain trajectories of up to 20,000 frames, reconstructing complex volumes of the ECS. We found a striking diversity of ECS dimensions down to 40 nm, as well as large variation of local viscosity values.

Robert Colbourn[1]*, J Goodman[2,3], S Hrabetova[4]
[1]School of Graduate Studies, State University of New York Downstate Medical Center, USA; [2]Institute for Basic Research in Developmental Disabilities, USA; [3]Department of Physiology and Pharmacology, Department of Neurology, State University of New York Downstate Medical Center, USA; [4]Department of Cell Biology, State University of New York Downstate Medical Center, USA

Dynamic Volume Changes of the Brain's Extracellular Space Underlying Seizures

The brain's extracellular space (ECS) is known to play a critical role in determining the excitability of neurons, and through this function, promote or inhibit seizure activity. It has been established that the ECS undergoes a long-lasting shrinkage of about 30% during a seizure. However, this project investigates a previously unreported phenomenon: the ECS undergoes a fast shrinkage, then slow expansion back to baseline volume during each synchronous neuronal discharge that occurs during epileptiform activity. The goal of this project is to establish these dynamic volume changes (DVCs) as a mechanism that promotes seizure activity and determine if manipulation of this phenomenon halts seizures. The first experiments that characterized DVCs during drug-induced epileptiform activity in mouse neocortical slices revealed that DVCs lead to a transient 22% reduction in ECS volume. This observation has also been repeated in vivo through rat neocortical measurements of ECS volume during drug-induced epileptiform activity. Because water transport between the intra- and extra-cellular compartment is likely responsible for DVCs, pharmacological blockade of osmotic and ionic transport proteins was performed, revealing several protein dependencies of seizures and their DVCs. These channels and others yet to be investigated may represent new therapeutic targets to treat seizures.

Scott Shippy*, P Fisher
Department of Chemistry, Laboratory of Integrative Neuroscience, University of Illinois at Chicago, IL USA

Miniaturized push-pull perfusion sampling of hippocampal slices

Techniques to sample the extracellular space provide a distinct compatibility for measurement of varied chemical content. The miniaturization of sampling probes will provide advantages both for both increased sampling spatial resolution and reduced damage to tissue. A miniaturized push-pull probe is described in this work that has a pulled tip size on the order of single microns. Concentric, fused-silica capillary-constructed push-pull probes are pulled in a home-built, flame-based, gravity puller to fabricate single micron tips. Tip size is measured with optical and electron microscopies. Infusion and withdrawal flows are calibrated for 10-20 nL/min perfusion rates similar to larger, low-flow push-pull perfusion probes. Probes are tested in mouse hippocampal slices to characterize glutamate content via a capillary electrophoresis assay. While miniaturized probe backpressures are somewhat increased compared to low-flow push-pull perfusion, vacuum withdrawal flow rates are easily realized. In vitro testing shows greater than 90% recoveries of standards amino acids. Ex vivo sampling from hippocampal tissues demonstrates low micromolar concentrations and a loss of glutamate from slices over time. Comparison of amino acid levels over 2 hours of sampling does not show evidence of tissue damage with probe placement. Experiments for combined sampling and electrophysiology are discussed.

Symposium 8: In Vitro and In Vivo Single Neuron Approaches to Study Neurodegeneration

Chair: Marie-Francoise Chesselet

Nader Pourmand
UC Santa Cruz, USA

Nanopipette technology for analysis of single living cells and subcellular compartments

Approaching sub-cellular biological problems from an engineering perspective begs for the incorporation of electronic readouts. With their high sensitivity and low invasiveness, nanotechnology-based tools hold great promise for biochemical sensing and single-cell manipulation. During my talk I will discuss the incorporation of electrical measurements into nanopipette technology and present results showing the rapid and reversible response of these subcellular sensors to different analytes such as antigens, ions and carbohydrates. In addition, I will present the development of a single-cell manipulation platform that uses a nanopipette in a scanning ion-conductive microscopy technique. We use this newly developed technology to position the nanopipette with nanoscale precision, and to sense, inject and/or aspirate a minute amount of cytoplasmic material to and from individual cells without comprising cell viability. Furthermore, I will discuss our strategy for a new, single-cell DNA/ RNA sequencing technology that will potentially use nanopipette technology to analyze the minute amount of aspirated cellular material.

J Linsley[1], D Linsley[2], **Steven Finkbeiner[1,3]***
[1]Gladstone Institutes UCSF, USA; [2]Brown University, Providence, RI, USA; [3]UCSF, USA

Development of a new biosensor and use of convolutional neural networks to reliably detect cell death in vitro and in vivo

Cell death is an important endpoint for normal developmental processes and in a variety of diseases. However, reliably measuring cell death remains challenging because the morphological changes can be ambiguous and mechanisms exist in vivo to quickly remove cell corpses, often before they can be detected. To address this need, we developed a new biosensor designed to detect elevated intracellular Ca^{2+} levels that are only achieved when cells are irreversibly committed to die. We modified a genetically encoded Ca^{2+} indicator, CEPIA, to engineer into it the appropriate sensitivity to elevated Ca^{2+} and validated it using a variety of mechanisms to induce death in cultured rodent neurons, human neurons differentiated from induced pluripotent stem cells, mouse hippocampal organotypic slice culture, and neurons of the zebrafish CNS in vivo. We then used the biosensor as ground truth and attempted to train convolutional neural networks (CNN) to determine the time of death of cells based on the morphological changes they exhibit. We could train CNNs to predict cell death in vitro from morphology information alone with 99.9% accuracy and in vivo with > 90%

accuracy. We conclude that we developed a useful new biosensor for accurately detecting cell death in vitro and in vivo and showed that it can be used to train CNNs to reliably detect cell death based solely on morphological changes, something humans have difficulty doing well.

Genevieve Rougon*, F Debarbieux
Institut des Neurosiences Timone CNRS, Marseille, France

Quantitative intravital imaging of the neuroimmune cellular interactions in the pathological central nervous system
Our objective is to analyze the interdependence of inflammation, neurodegeneration and disease progression in pathologies of the central nervous system. A major aim is to identify subpopulations of resident (activated microglia) and recruited peripheral immune cells and their dynamic interplay that regulates the fate of neuronal networks.
We will describe the methodological developments of spectral two photon imaging we have implemented allowing access to single cell dynamics in 3D in the spinal cord of living heterozygous Thy1-CFP//LysM-EGFP//CD11c-EYFP reporter mice in which neurons (CFP), myeloid cells (EGFP) microglia (EYFP) and blood vessels (Q-dots 655) fluoresce in different colors. We will show that after induction of autoimmune experimental encephalitis (EAE) in these mice recurrent imaging allowed to characterize the distribution and quantify the cell densities of identified cell subsets in the same region of interest throughout disease progression. Briefly, we identified a stepwise innate immune program with neutrophils and P1 monocytes invading the spinal cord through the meninges. Recruitment of these cells resulted in a progressive gradient of plaques development from the superficial white matter towards deeper areas, contrary to microglial activation. Maturation of P1 towards P3 monocyte-derived dendritic cells in plaques marked a stabilization of the axonal degradation and clinical signs.

B Sun[1], M Wang[2], B LI[2], M Booth[1], C Xu[3], **Francis Szele[1]***
[2]Cornell University, [3]Ithica New York; [1]University of Oxford

Imaging neurogenesis and cancer in the mammalian brain
Postnatal and adult mammalian neurogenic niches are excellent systems for mechanism discovery and have therapeutic implications in treatment of disease but can also become harmful by giving rise to cancer stem cells. A central problem in studying stem cell dynamics and migration of progeny in the subventricular zone and subgranular zone is that they are deep in the brain, out of reach of confocal and 2-photon microscopy. We are developing 3-photon to see down to 1.5 mm below the surface of the brain in live animals. Long wavelength (1700 nm) light has minimal scattering and permits detection of red fluorescent flourochromes that labeled SVZ and SGZ cells. We have developed a model of neurogenic niche gliomagenesis by conditionally introducing the IDH1R132H mutation into the SVZ and SGZ. We aim to test the dual hypothesis that IDH1R132H induced gliomagenesis infiltrates by increased mitosis when induced in stem cells but infiltrates by migration when induced in neuroblasts. We also hope to combine these approaches of cellular imaging with molecular imaging.

Charmaine Lang*, K Campbell, B Ryan, C Webber, R Wade-Martins
Oxford Parkinson's Disease Centre, Department of Physiology, Anatomy and Genetics, University of Oxford, UK

Single cell sequencing reveals HDAC4 as a regulator of cellular phenotypes in Parkinson's iPSC-derived dopamine neurons
Patient obtained induced pluripotent stem cell (iPSC)-derived dopamine neurons provide an opportunity to model Parkinson's disease (PD) in previously inaccessible neurons, which recapitulate the genetic background of patients. However cell cultures are notoriously confounded by cellular heterogeneity. By isolating dopaminergic neurons from these cultures and applying high-resolution single cell and bulk RNA-sequencing transcriptomic analyses to PD iPSC-derived dopamine neurons, we exploited intra-culture cellular heterogeneity to identify a progressive axis of gene expression variation within the dopaminergic neuron cell population. Analysis of genes differentially expressed (DE) early across this axis identified the transcriptional repressor, histone deacetylase 4 (HDAC4), as an upstream regulator of disease progression. HDAC4 was observed as mislocalised to the nucleus in PD iPSC-derived dopamine neurons and repressed genes early in the disease axis, leading to later deficits in ER stress. Treatment of neurons with compounds, re-localised HDAC4, activated previously repressed early DE genes and corrected a number of PD-related cellular phenotypes, including ER stress, autophagy perturbation and alpha synuclein release. Our study demonstrates how

single cell RNA-Sequencing can exploit iPSC cellular heterogeneity for patient stratification and to reveal disease mechanisms, which can be used to identify potential targets and repurposed therapeutics of interest in the treatment of PD.

Symposium 9: Nitric Oxide Signaling from Molecule to Brain

Chairs: Stephane Marinesco, Anne Meiller

E Eroglu[1], H Bischof[1], B Gottschalk[1,2], F Hellal[3], M Rehberg[3], M Waldeck-Weiermair[1], N Plesnila[3], WF Graier[1,2], **Roland Malli[1,2]***

[1]Institute of Molecular Biology & Biochemistry, Medical University of Graz, Austria; [2]BioTechMed Graz, Austria; [3]Ludwig-Maximilians University, Institute for Stroke and Dementia Research, University of Munich Medical Center, Germany

Shining Light on Cellular Nitric Oxide and Potassium Signals Using Genetically Encoded Probes

Diverse signalling molecules and ion fluxes control the activity and functions of neurons. Among many factors, nitric oxide (NO) and potassium ions (K^+) have profound impacts on neuronal signalling. However, neither the short-lived NO radical nor K^+ signals can be easily detected on the level of individual cells and cellular compartments. We have recently developed genetically encoded fluorescent probes that enable real-time imaging of either NO or K^+ dynamics with high spatial and temporal resolution. The novel NO probes, referred to as geNOps, consist of a specific NO binding domain, which is fused to different fluorescent protein variants. Upon NO binding, fluorescence is immediately quenched, allowing real-time recordings of subcellular NO dynamics. The geNOps technology has been used to visualize cellular NO signals in response to diverse NO-liberating compounds and to detect endogenous NO formation. Currently, the usability of geNOps for in vivo NO imaging is tested. Very recently, we have developed the first genetically encoded K^+ probes, referred to as GEPIIs (genetically encoded potassium ion indicators). The GEPIIs enable real-time imaging of intra- and extracellular K^+ dynamics. As GEPIIs are functional in vivo, these probes might open a new era of K^+ imaging in the brain of living animals.

Mark Schoenfisch*, MD Brown

Department of Chemistry, University of North Carolina at Chapel Hill, USA

A durable, permselective nitric oxide electrochemical sensor for continuous, in situ monitoring of macrophage activity

Electrochemical techniques make possible the continuous measurement of relevant electroactive species in situ. Applying these techniques to biological environments comes with a unique set of challenges, particularly biofouling-related deterioration of the transducer surface and the presence of interferent species. Herein, we detail the fabrication of an electrochemical cell and original sensor for direct, long-term (i.e., >24 h) monitoring of nitric oxide (NO) as a surrogate for macrophage activity. The sensor was fabricated using a macrodisc platinum electrode modified with poly(5-amino-1-naphthol) and a fluorinated xerogel. This composite design led to high sensitivity and selectivity for NO with sustainable analytical performance over successive trials in proteinaceous media. A custom well-plate cover was implemented to support 8 such sensors for multiplexed monitoring of NO from cultured cell populations. Macrophages were then exposed to different stimulation conditions (i.e., neutral, pro-inflammatory, and inhibited) to better understand the time-dependence of macrophage activity.

Anne Meiller[1]* and S Marinesco

[1]INSERM U1028, CNRS UMR5292; Lyon Neuroscience Research Center, AniRA-Neurochem Technological Platform, Lyon, France; [2]Université Claude Bernard Lyon 1, Lyon, France

In vivo brain nitric oxide detection using fluorinated xerogel-coated carbon fiber microelectrodes.

Nitric oxide (NO) is an important free radical synthetized and released by brain cells. It can modulate synaptic transmission and neuronal network activity but also mediate neuronal injury through oxidative stress. It is believed that NO exerts its physiological functions at low concentrations whereas oxidative stress occurs at much higher levels.

However, the quantitative threshold at which NO concentrations become toxic is still poorly defined. Here, we detected endogenous brain NO release using 7 µm diameter carbon fiber microelectrodes first coated with a layer of nickel-porphyrin (Ni-P) and a screening layer composed of trimethoxymethylsilane and Heptadecafluoro-1,1,2,2-tetrahydrodecyl trimethoxysilane. The fluorinated xerogel improved the selectivity of the sensor compared to Nafion, a fluoropolymer commonly used to block interfering molecules. Nitrite, 5-HT and AA amperometric detection was significantly reduced by the silane layer and NO detection was stable over 7 days of storage and throughout a 3h in vivo experiment. In vivo, these electrodes could quantify brain NO release evoked by a toxic local microinjection of the glutamatergic agonist N-Methyl-D-aspartate at 1.33 [0.49-4.93] µM. The amperometric signal was almost completely blocked by the NO-synthase inhibitor 7-nitroindazole. Fluorinated xerogel-coated carbon fiber microelectrodes therefore provide excellent stability, sensitivity and selectivity to detect brain NO and quantify its concentrations. Toxic NO actions like those evoked by the neurotoxin NMDA may take place in the low micromolar range.
Supported by CNRS, Inserm, University of Lyon., and grant FGC46-2016 from Fondations Gueules Cassées.

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Nitric oxide signalling in corticostriatal circuits: implications for the treatment of Huntington's disease
Nitric oxide (NO) is a gaseous neurotransmitter released by interneurons in corticostriatal circuits. NO activates soluble guanylyl cyclase (sGC) in cortical and striatal projection neurons, leading to intracellular elevations in cGMP. NO, cGMP and downstream effector targets (e.g., protein kinases, phosphodiesterases (PDEs)) play key roles in synaptic integration and neuroplasticity in corticostriatal circuits. Using amperometry and histochemistry, we have shown that corticostriatal transmission activates neuronal NO synthase (nNOS)-containing interneurons via an NMDA and dopamine D1/5 receptor-dependent mechanism. In vivo electrophysiological studies demonstrated that stimulation of nitrergic transmission facilitates the excitatory effects of glutamatergic transmission on striatal projection neurons. Interestingly, this "feed-forward" excitatory influence of NO-cGMP signalling on corticostriatal transmission is compromised in transgenic nNOS knockout mice and rodent models of Huntington's disease (HD). Studies in the z_Q175 mouse model of HD showed that nNOS expression and activity is down-regulated in corticostriatal circuits in an age- and sex-dependent manner which is associated with deficits in cortical activation of striatal projection neurons. Moreover, these deficits in corticostriatal transmission were rescued following restoration of cGMP signalling using selective PDE inhibitors. Thus, pharmacological activation of NO-cGMP signaling could be useful for restoring corticostriatal transmission and associated motor and cognitive deficits observed in HD.

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[1]Department of Neurobiology, Silberman Institute of Life Sciences, The Hebrew University, Jerusalem, Israel; [2]The Ruppin Academic Center, School of Marine Sciences, Michmoret, Israel

Long-term potentiation (LTP) expression and maintenance in the octopus vertical lobe is mediated by long-term elevation in nitric oxide (NO) concentration
The octopus vertical lobe (VL), a brain area that controls the sophisticated learning of this invertebrate, demonstrates a robust activity-independent NMDA-independent LTP. We show here that the presynaptic expression of LTP involves activation of nitric oxide synthase (NOS) and that nitric oxide (NO)-dependent reactivation of NOS functions as a 'molecular switch' mediating the very long, protein synthesis-independent, LTP maintenance (>10h). While NADPH-diaphorase histochemistry supports the presence of NOS in the VL, we could not find any indication for the involvement of NO-dependent cGMP cascade in LTP. Additionally, NO-donors and NO-scavengers had no effect. These negative results suggest the possible involvement of processes that function at high NO concentration (e.g., s-nitrosylation).Therefore, we measured NO concentration amperometrically and found that induction of LTP is accompanied by a long-term increase in the amperometric signal that corresponded with the oxidation potential of NO (750 mV). The increase was to around µM ranges; much higher than that found for the activation of the cGMP cascade. We therefore hypothesize that a process such as s-nitrosylation could serve as an effective mediator of a local retrograde message for ensuring specificity in presynaptic LTP.
Support: ISF, BSF and the National Inst. For Psychobiology (to TM and NN)

Symposium 10: Bad Things Happen: The Role of Phasic Dopamine Signaling in Learning About, and Responding to, Negative Stimuli

Chairs: Eleanor Simpson, Mitch Roitman

Abigail Kalmbach
Columbia University, New York State Psychiatric Institute, USA

Knowing when to stop: dopamine encoding of inhibitory cues in the ventral striatum

The role of dopamine (DA) signalling is unclear during inhibitory learning, which is when an animal learns to refrain from responding. Therefore, we examined DA signalling in the ventral striatum of mice during inhibitory conditioning. Animals learned to suppress reward-directed behaviour when the inhibitory conditioned stimulus (a tone), is present. Using fast scan cyclic voltammetry, we found that DA encodes inhibitory cues. Specifically, we observed a slow decrease in DA at tone onset but a rapid return to baseline DA levels at tone offset, when rewards are again available. Thus, we hypothesize that the mechanism that suppresses DA initially is physiologically different from the mechanism by which DA rebounds at the termination of the tone. Interestingly, the inhibition of behaviour and of DA signalling have similar onset dynamics but become disjointed later in the stimulus when the lever pressing gradually ramps back to baseline levels yet the DA level remains low until tone offset when DA quickly returns to baseline. Accordingly, we hypothesize that DA does not instruct all aspects of behaviour in this paradigm. Our findings could provide better understanding of behavioural deficits in inhibiting action observed in some psychiatric conditions, including ADHD and schizophrenia.

K Yu, S Ahrens, X Zhang, **Bo Li***
Cold Spring Harbour Laboratory, USA

The amygdala circuits in the regulation of aversive learning

The amygdala is essential for learning and expression of behavioral responses driven by either reward or aversive stimuli. How exactly distinct amygdala circuits contribute to the generation of such divergent behavioral responses remains unclear. Our recent studies in mice indicate that aversive stimulus-driven learning induces distinct plastic changes in distributed amygdala circuits, including those in the central amygdala (CeA). Furthermore, by using optogenetics, chemogenetics, electrophysiology, and in vivo GCaMP6 imaging methods, we recently found that the PKC-δ-expressing CeA neurons are essential for the synaptic plasticity underlying learning in the lateral amygdala (LA), as they convey a teaching signal to LA neurons during fear conditioning, likely through midbrain neuromodulatory systems. Here I will report our recent findings regarding the cellular and circuit mechanisms underlying some of the behavioral roles of the amygdala.

Erik Oleson*, SA Schelp, KJ Pultorak
University of Colorado, Denver, CO, USA

A transient dopamine signal represents avoidance value and causally influences the demand to avoid

The canonical view of mesolimbic dopamine is that this reward molecule guides positive reinforcement. While the avoidance of harmful stimuli is similarly pertinent to an organism's survival, the role of dopamine in avoidance (i.e., negative reinforcement) remains controversial. In the present study, after finding that dopamine concentration scales with price in an economics-based footshock avoidance task, we assessed causality by optogenetically increasing release at either an avoidance predictive cue or upon successful avoidance. As in the field of economics, price sensitivity was assessed using demand curves. Increasing release at cue made animals more sensitive to price, consistent with a negative prediction error. Increasing release at avoidance made animals less sensitive to price, consistent with a positive prediction error. These data demonstrate that transient dopamine release events can represent the value of avoidance outcomes and capably modify the demand to avoid. Our new avoidance data will be compared and contrasted to those recently reported using an economics-based sugar seeking task.

Matthew Roesch
University of Maryland College Park, USA

Dopamine signaling in social contexts: when good and bad things happen to oneself and others
Dopamine (DA) release in nucleus accumbens core (NAc) increases and decreases in response to events that are better or worse than expected, respectively. In the appetitive domain, cues that predict reward or uncertain delivery of reward increase DA release, and cues that predict less favorable reward and omission of expected reward decrease DA release. In aversive contexts, cues predictive of unavoidable aversive events (e.g., quinine, air puff, shock) or delivery of those aversive outcomes themselves reduce DA release, whereas unexpected omission of aversive events or the cues that predict avoidable shock elicit phasic DA release. Few studies have examined DA release in the context of both appetitive and aversive cues, and even fewer have examined how appetitive and aversive events directed to a conspecific, instead of oneself, alter DA release. Here we show that DA release (Fast Scan Cyclic Voltammetry) within a single microenvironment is higher for reward and avoidance cues compared to neutral cues. Further, in three different social paradigms we show that DA release is modulated by appetitive and aversive events that occur to a conspecific located nearby and that both behavior and DA release better reflected the value associated with benefiting oneself as opposed to the conspecific.

Evgeny Budygin
Department of Neurobiology and Anatomy, Wake Forest School of Medicine, Winston Salem, NC, USA

Exploring phasic changes in striatal dopamine release under the effect of negative stimuli
The perception and consequences of pleasurable and negative stimuli are different, and the underlying substrates mediating these opposing phenomena are unclear. To address the question of how dopamine (DA) neurotransmission within the striatum encodes negative stimuli, we evaluated DA dynamics in response to aversive stimuli using voltammetry. Surprisingly, we found that the neurochemical and anatomical substrates responsible for the perception and processing of pleasurable, rewarding stimuli were activated by tail pinch. Thus, tail pinch triggered transient increases in extracellular DA concentration in the nucleus accumbens (NAc) of freely moving rats. These transients did not differ from those triggered by rewarding stimuli. To avoid the influence of other possible stimuli, striatal DA responses were explored in anesthetized rats. We found that pinch-evoked DA release in the dorsal striatum and NAc core was time locked to the duration of the stimulus. However, DA was released in the NAc shell only when tail pinch was terminated. Furthermore, it was revealed that unpainful tail touch could also induce DA transients but only if it was previously coupled with the painful stimulus. Our data suggest the existence of some overlap in the neurochemistry of perception and processing of negative and positive stimuli within striatum.extracellular DA concentration in the nucleus accumbens (NAc) of freely moving rats. These transients did not differ from those triggered by rewarding stimuli. To avoid the influence of other possible stimuli, striatal DA responses were explored in anesthetized rats. We found that pinch-evoked DA release in the dorsal striatum and NAc core was time locked to the duration of the stimulus. However, DA was released in the NAc shell only when tail pinch was terminated. Furthermore, it was revealed that unpainful tail touch could also induce DA transients but only if it was previously coupled with the painful stimulus. Our data suggest the existence of some overlap in the neurochemistry of perception and processing of negative and positive stimuli within striatum.

Symposium 11: A Reversal of Fortune for Peptides and Endocannabinoids: from Poor Cousins to Rich Regulators of Brain Microcircuits

Chairs: Margaret Rice, Anushree Karkhanis

DL Bernstein, JK Shaw, ZD Brodnik, EM Black, Ilse Alonso, **Rodrigo España***
Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA, USA

Hypocretin / Orexin Influences Dopamine Neurotransmission and Cocaine-Associated Behavior
The hypocretin / orexin system influences reward and reinforcement processes via actions on the mesolimbic dopamine system. Using a combination of behavioral and neurochemical techniques we have demonstrated that the hypocretin

system impacts cocaine-induced alterations in reward/reinforcement behavior as well as dopamine signaling in the nucleus accumbens core. Across various schedules of reinforcement, we have demonstrated that treatment with a hypocretin peptide promotes cocaine self-administration and enhances dopamine responses to cocaine. In contrast, disruptions in hypocretin neurotransmission reduce cocaine self-administration and attenuate cocaine-induced elevations in dopamine. These effects appear to be specific to actions on dopamine neurons in the ventral tegmental area given that dopamine specific knock down of hypocretin receptor 1 in this region recapitulates many of the effects observed with previous pharmacological approaches. When considered together, these observations demonstrate that the hypocretin system participates in the regulation of dopamine signaling under baseline conditions and in response to cocaine and provide additional evidence for the hypothesis that the hypocretins are involved in reward and reinforcement processes through actions on the mesolimbic dopamine system.

Michael Beckstead*, CW Tschumi
Aging & Metabolism Research Program, Oklahoma Medical Research Foundation, Oklahoma City, USA

Actions of the modulatory peptide neurotensin on inhibitory input to midbrain dopaminergic neurons

Dopaminergic neurons in the ventral midbrain receive inhibitory input from a variety of sources. In brain slices from mice, electrical or optical stimulation of either GABA or dopamine input elicits inhibitory postsynaptic currents in dopaminergic neurons. The ability to measure these synaptic events using patch clamp electrophysiology allows us to explore the ability of modulatory neuropeptides to induce plasticity of inhibitory neurotransmission. Neurotensin is a peptide widely implicated in modulation of dopaminergic behaviors, and we have discovered that neurotensin modulates inhibitory input to dopaminergic neurons both pre- and postsynaptically. Neurotensin depresses D2 dopamine autoreceptor signaling in a manner that is persistent and involves NTS2 receptors. Neurotensin also inhibits GABAB receptor-mediated input through NTS2 receptors, but that effect is transient and exclusively postsynaptic. Surprisingly, neurotensin also increases the readily releasable pool of GABA at fast GABAA synapses through NTS1 receptors, resulting in a net speeding of inhibitory input. To further investigate the physiology of endogenously-released neurotensin we are now coupling transgenic mice with optogenetic techniques. Our findings thus far suggest that neurotensin released from dopaminergic neurons depresses dopamine signaling to a greater extent than neurotensin released from non-dopaminergic neurons, and may indicate that physiological neurotensin acts in a synapse-localized fashion.

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Department of Neurosurgery[1], Neuroscience Institute[2], Neuroscience & Physiology[3], New York University School of Medicine, USA

Peripheral peptides insulin and leptin target striatal cholinergic interneurons to enhance dopamine release

The peripheral peptides insulin and leptin cross the blood-brain barrier to act as satiety signals in the hypothalamus, thereby regulating feeding behavior. Receptors for insulin are also found in the striatum, where insulin can alter extracellular DA concentration ($[DA]_o$). We have employed a variety of neurochemical tools to probe mechanisms underlying the effects of insulin. Using fast-scan cyclic voltammetry (FSCV) to monitor evoked $[DA]_o$ and DA transporter (DAT) kinetics in striatal slices, we discovered that physiological concentrations of insulin enhance evoked $[DA]_o$, despite increasing DAT activity, via PI3-kinase. Immunohistochemistry indicates that striatal cholinergic interneurons (ChIs), as well as DA axons, express insulin receptors. Patch-clamp recording shows that insulin increases ChI excitability; this enhances evoked ACh release detected by enzyme-coated carbon-fiber microelectrodes and FSCV. Consistent with the ability of ACh from ChIs to facilitate axonal DA release via nicotinic ACh receptors (nAChRs), insulin's effects are lost when nAChRs are blocked or when evoked $[DA]_o$ and ChI excitability are examined in mice lacking forebrain ACh. Interestingly, we recently found that the hormone leptin, acting via similar signaling pathways, also enhances ChI excitability and evoked $[DA]_o$. Thus, ChIs act as a hub through which these peripheral peptides can regulate local striatal circuits.

Y Mateo[1], KA Johnson[1], DP Covey[2], BK. Atwood[1], HL Wang[3], S Zhang[3], I Gildish[2], R Cachope[2], L Bellocchio[4], M Guzmán[4], M Morales[3], DM. Lovinger[1], **Joseph Cheer**[2,5]*
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of Maryland School of Medicine, Baltimore, Maryland; [3]Neuronal Networks Section, National Institute on Drug Abuse, US National Institutes of Health, Baltimore, Maryland, USA; [4]Department of Biochemistry and Molecular Biology I, Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) and Instituto Universitario de Investigación Neuroquímica (IUIN), Complutense University, 28040 Madrid, Spain; [5] Department of Psychiatry, University of Maryland School of Medicine, Baltimore, Maryland

Endocannabinoids on cortical terminals orchestrate local modulation of dopamine release in the nucleus accumbens

Dopamine (DA) transmission mediates numerous aspects of behavior. Although DA release is strongly linked to firing of DA neurons, recent developments indicate the importance of presynaptic modulation at striatal dopaminergic terminals. The endocannabinoid (eCB) system regulates DA release and is a canonical gatekeeper of goal-directed behavior. Here we report that extracelluar DA increases induced by selective optogenetic activation of cholinergic neurons in the nucleus accumbens (NAc) are inhibited by CB1 agonists and eCBs. This modulation requires CB1 receptors on cortical glutamatergic afferents. DA increases driven by optogenetic activation of prefrontal cortex (PFC) terminals in the NAc are similarly modulated by activation of these CB1 receptors. We also demonstrate that this same population of CB1 receptors is required for optical self-stimulation sustained by PFC afferents in the NAc. These results establish local eCB actions within the NAc that inhibit mesolimbic DA release and contribute to reward-driven behavior.

Anushree Karkhanis*, JL Weiner, SR Jones
Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC, USA

Adolescent social isolation augments kappa opioid receptor function in the nucleus accumbens and basolateral amygdala of rats

Adverse experiences during adolescence increase alcohol use disorder vulnerability during adulthood in humans. Rats exposed to adolescent social isolation (aSI) show greater ethanol intake in adulthood compared to group housed (aGH) controls. Acute stress elevates dynorphin levels, a kappa opioid receptor (KOR) ligand, and KOR activation inhibits dopamine release in the NAc and the BLA, two interconnected regions integral in stress and reward-seeking behavior. Baseline dopamine levels were lower in the NAc and BLA of aSI rats and were reversed following KOR-inhibition. Ethanol-induced dopamine elevations were greater in NAc and BLA of aSI rats. KOR-inhibition augmented ethanol-induced dopamine responses in the NAc but attenuated them in the BLA of aSI rats. The inhibitory effects of KOR-activation on dopamine release were enhanced in the NAc of aSI rats suggesting that chronic stress augments KOR function. KOR-overexpression in dopamine neurons augmented ethanol intake in aGH rats. KOR-inhibition differences may explain ethanol's effects on behaviors related to specific brain regions, e.g., ethanol-induced augmented dopamine in the NAc increases reinforcement, whereas augmented dopamine in the BLA may reduce heightened anxiety levels. Therefore, KOR inhibition in the NAc attenuates reinforcing value and reduces anxiety decreasing effects of ethanol in addition to decreasing overall anxiety.

Symposium 12: Towards Microdialysis 2.0 – a Faster, Smaller, Smarter Microdialysis for Neurochemical Monitoring

Chairs: Martyn Boutelle, Steve Weber

Stephen Weber*, AC Michael, KT Ngo, A Jaquins-Gerstl, RA Wilson, MT Rerick
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Improving microdialysis/online liquid chromatography capability: Higher time resolution for dopamine and serotonin and peptide quantitation in dialysate

Time resolution of microdialysis has historically been in the many minutes up to one hour range. We are attempting to improve that in order to obtain more information from microdialysis measurements in brain in vivo. Currently, we are focused on online measurements of dopamine and serotonin in dialysate by fast capillary liquid chromatography (cLC). The time resolution in the microdialysis system itself – the probe and connecting tubing – will become the limiting factor as the dialysate analysis speed improves. Time resolution is characterized by a standard deviation corresponding to a Gaussian profile that results from an impulse input. Currently, the time standard deviation of the microdialysis system

is about 15 s which we hope to improve. Using the fast cLC system, we have characterized K^+-induced oscillations in DA in dialysate from striatum which we attribute to spreading depolarization. We have also investigated the effect of a four-day dexamethasone retrodialysis paradigm on striatal DA in dialysate. Separately, differences in peptide activity in brain in vivo has become interesting to us. This requires accurate online quantitation of peptides by microdialysis. We are developing an online isotopic labelling procedure and applying it to leucine-enkephalin in the hippocampus with online cLC/MS.

Martin Eysberg
Antec Scientific, Netherlands

Method development in neurotransmitter analysis to improve selectivity, sensitivity and robustness
In vivo microdialysis has become an invaluable tool that provides real-time information of neurotransmitter levels in living brain. Microdialysis samples are collected and stored, or analyzed immediately in an on-line configuration using UHPLC-ECD. It is an analytical challenge to provide reproducible and accurate data as neurotransmitter levels are often below picomolar concentration range.
To get as much information from samples <5ul, improvement of selectivity, sensitivity and robustness is necessary. To improve and obtain this, chromatography aspects e.g. high efficiency columns, flow cell configurations, etc are studied and evaluated.
The ALEXYS Neurotransmitter analyzer comprises a number of integrated system solutions, which have been developed for trace analysis of neurotransmitters. Parallel and serial detection schemes using multiple flow cells have been used instead of running sequential trials for different neurotransmitters. Dual or triple loop injection valves are applied with minimum sample consumption on parallel UHPLC systems under completely different conditions. Getting more information out of fewer samples is not only saving time and money but - in the end - also rodents.

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[1]JOANNEUM RESEARCH Forschungsgesellschaft mbH – HEALTH; [2]Medical University of Graz, Div. Endocrinology Metabolism, Dep. of Internal Medicine; [3]Bioanalytical Systems Inc., (BASi)

Cerebral open flow microperfusion – sa sampling tool for longerm monitoring of transport across the BBB
A recently developed sampling technique called cerebral open flow microperfusion (cOFM) enables sampling of brain interstitial fluid and CSF and provides long term access directly to the brain tissue bypassing the BBB. cOFM probes feature macroscopic openings for the exchange of substances with the cerebral ISF. This open exchange structure avoids membrane-related problems such as biofouling, protein clotting, high molecular weight cut-off and the exclusion of large and lipophilic substances. Similar to other probe-based sampling techniques, cOFM probe implantation causes capillary rupture and thus disruption of the BBB. As an intact BBB is necessary to assess substance transport across the BBB, cOFM studies implement a 14 day healing period to ensure re-establishment of the BBB. Most probe-based sampling technologies that use implanted probes for measurement and sampling in the brain are limited in application time due to the formation of a glial scar that leads to encapsulation of the probe. The design of the cOFM probe and especially the used materials are optimized to evoke minimal tissue reaction. No continuous glial scar was found up to 30 days after cOFM probe implantation when glial scarring was measured by qualitative and quantitative histological tissue analysis of microglia and astrocytes.

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Multiplexed serotonin and dopamine monitoring: Why faster is better v2.0
In 1949, Donald Hebb coined the phrase “neurons that wire together fire together”. While Hebb was likely referring to neurons in local microcircuits, this principle can also be applied to long range projections. Anatomical and physiological studies show that serotonergic and dopaminergic neurons are “wired together” in brain regions including the ventral tegmental area, striatum, and frontal cortex. However, most neurochemical methods currently suffer from limitations involving the detection of one neurotransmitter at a time. One advantage of microdialysis is that since dialysate samples are analyzed ex vivo using separation followed by detection, multiple neurotransmitters can be investigated

simultaneously to understand their interactions. These interactions are important for constructing holistic pictures of brain function. In the past, multiplexed measurements by microdialysis came at the expense of temporal resolution. Recently, significant improvements in microdialysis sampling, separation speeds, and detection limits have enabled the investigation of multiple neurotransmitters with temporal resolution in the minute-to-minute range (and faster in some cases). We have focused on differential detection of serotonin and dopamine. Using "fast" microdialysis, as well as other methods (*e.g.*, fast-pulse voltammetry, aptamer-FET sensors), we observe that simultaneous measurements of serotonin and dopamine often lead to findings in pharmacologic and genetic animal models that might not be arrived at when focusing on a single neurotransmitter.

Alberto Morales*, K Pardo-Peña
Laboratorio de Neuroquímica, Molecular and Cellular Department, University of Guadalajara, Guadalajara, Jalisco, México

Glutamate measurement online and at high temporal resolution, using a new microdialysis procedure and an optic device

The classic microdialysis method lacks of high temporal resolution and is mainly coupled to HPLC. In this work, a new alternative is proposed to improve this drawback and thus obtain a better understanding of the dynamics of Glutamate or other molecules of biological interest. The new set up was designed and built to measure hydrogen peroxide as a product of reaction of Glutamate oxidase, which is quantified by the Amplex Red method. The device consists in a fluorescence cell in which the fluid coming from the microdialysis is mixed online with an enzymatic reactor containing Glutamate oxidase and Amplex Red, then this mixture goes into the cell and the fluorescence is measured at sub-seconds time resolution, which is proportional to the Glutamate concentration. To test the reliability of this method, calibration curves for Glutamate were run resulting in a linear response ($R \geq 98$) and then a microdialysis probe was placed into the hippocampus to measure the Glutamate during seizure induced by pentilenetetrazole, the results showed a relation between the extracellular increase in this neurotransmitter and the seizure activity. This method can be used to measure other compounds that generate fluorescence with Amplex Red or any other fluorescence probe.

Robert Kennedy*, T Nguniverstagul, T White, D Steyer, A Valenta
Department of Chemistry, University of Michigan, Ann Arbor, MI, USA

Microfabricated Sampling Probes: Challenges and Opportunities

Microdialysis sampling is a convenient way to monitor a wide range of neurochemicals in vivo and to perform highly multiplexed monitoring. However, the large size and low temporal resolution compared to sensors and imaging methods are significant drawbacks that have limited more usage. We describe recent advances in using microfabrication methods to make microdialysis and low-flow push-pull perfusion probes. With microfabrication in silicon, probes with 50-80 um width and 25-40 um thickness can be made. These small probes provide much better spatial resolution than conventional sampling probes. Several challenges must be overcome to allow their widespread use. Probe fabrication and packaging must be simplified. Perhaps more importantly, the very low flow rates (< 100 nL/min) demand new methods of fraction collection and analysis. We had demonstrated fraction collection as droplets with nanoliter volume, corresponding to seconds temporal resolution. Direct infusion into a mass spectrometer is proving to be a simply way to analyse the droplets. Early work suggests potential to monitor acetylcholine, glutamate, and GABA by this method. Evidence suggests other neurotransmitters will also be detectable as well. The combined system allows versatile detection of many neurotransmitters with high temporal and spatial resolution in vivo.

Symposium 13: Expanding the Reach of Voltammetry Beyond Dopamine

Chairs: Jill Venton, Sara Jones

Zoe McElligott*, ES Cogan, KT Schmidt
Bowles Center for Alcohol Studies, Departments of Psychiatry and Pharmacology, UNC Chapel Hill, USA

Optogenetics-assisted Fast-Scan Cyclic Voltammetry for the Detection of Serotonin and Norepinephrine

Fast-scan cyclic voltammetry (FSCV) is an electrochemical technique that is optimized for detecting dopamine (DA) in regions of the brain with dense dopaminergic innervation, and low levels of innervation of other biogenic amines. Thus, the vast majority of studies have focused on measuring DA release and uptake in the dorsal and ventral striatum. Indeed, FSCV using the extended wave-form is ideal for the detection of DA over other biogenic amines, complicating measuring these neuromodulators in areas with mixed innervation. Here, we demonstrate that coupling FSCV detection to genetically defined, cell type specific optogenetic activation (using Channelrhodopsin – ChR2) allows for the measurement of both serotonin (5-HT) and norepinephrine (NE) in slices containing the bed nucleus of the stria terminalis (BNST) and the basolateral amygdala (BLA). Moreover, we establish that we are able to see ChR2 mediated release of both 5-HT and NE at stimulus trains with physiologically relevant tonic and phasic frequencies. These developments will allow for more detailed study of the mechanisms that govern 5-HT and NE release and uptake, and how they are modulated in models of neurological and psychiatric disorders.

Kenneth Kishida
Department of Physiology and Pharmacology; Department of Neurosurgery; Department of Biomedical Engineering, Wake Forest School of Medicine, Winston-Salem, NC, USA

Simultaneous Detection of Dopamine, Serotonin, and Norepinephrine using a Machine Learning-based approach to Fast Scan Cyclic Voltammetry

Fast scan cyclic voltammetry (FSCV) experiments in conscious humans undergoing brain surgery presents challenges to traditional methods of analyzing FSCV data. We sought a new approach to detect and estimate the concentration of dopamine that did not rely on the qualitative assessment of the background subtracted voltammogram and could be applied in settings were electrical stimulation and pharmacological manipulation of the targeted brain tissue was not possible. We employed a modern machine learning approach (the "elastic net") to train cross-validated, multivariate, penalized linear regression models to estimate and discriminate absolute dopamine levels from changes in pH. We have now extended this approach to simultaneously detect and discriminate dopamine, serotonin, and norepinephrine. I will discuss how prior assumptions about limitations and challenges to the FSCV method are specific to the traditional methods of analysis as it is routinely performed and not inherent to the voltammetric measurement itself. I will also discuss how to implement our approach and some of the potential pitfalls to avoid during implementation. Finally, I will introduce a new voltammetry protocol, which when combined with the machine learning based approach to detect and estimate neurotransmitter levels can increase the temporal resolution of voltammetry measurements by at least 10-fold.

Jill Venton*, M Ganesana, Y Wang, S Lee, J Borgus, M Nguyen
Department of Chemistry and Neuroscience Graduate Program, University of Virginia, Charlottesville, VA, USA

Mechanism and function of spontaneous adenosine transients

Adenosine is a ubiquitous signaling molecule in the brain that serves to modulate neurotransmission and cerebral blood flow. Traditionally, adenosine is thought to be a neuromodulator because it originates in many cell types, acts on a broad array of neurons, and accumulates on a slow time scale during diseases. Our lab developed fast-scan cyclic voltammetry at carbon-fiber microelectrodes for rapid adenosine measurements and we discovered a rapid mode of adenosine signaling that has many characteristics of both neurotransmission and neuromodulation. Spontaneous, transient adenosine release is observed in the caudate-putamen and prefrontal cortex of anesthetized rats. The transients last only 3-5 seconds, much more rapid than adenosine was thought to signal previously. Spontaneous transients are observed in anesthetized rats at a frequency of about a transient every 5 minutes and are 0.3 µM on

average. Mechanosensitive release is also observed and we verified our FSCV results with enzyme sensors. Transient adenosine has been shown to modulate phasic dopamine release and rapid changes in oxygen levels in vivo. Current experiments are exploring the role of transient adenosine in ischemia. Funding: NIH R01NS076875

Lanqun Mao
Beijing National Laboratory for Molecular Sciences, Key Laboratory of Living Biosystems, Institute of Chemistry, the Chinese Academy of Sciences, Beijing, China

In Vivo Electrochemistry to Understand Physiological Roles of Ascorbate

To understand the molecular basis of brain functions, researchers would like to be able to quantitatively monitor neurochemicals in vivo. However, the chemical and physiological complexity of the central nervous system (CNS) presents challenges for the development of these analytical methods. We used the redox nature of neurochemicals at the electrode/electrolyte interface to form a basis for selectively monitoring neurochemicals. In this presentation, I would introduce the recent process in our group on in vivo monitoring ascorbate in rat brain.

1) Carbon nanotubes (CNTs) provide an electrode/electrolyte interface for the selective oxidation of ascorbate and, based on this, we have developed both in vivo voltammetry and an online electrochemical detecting system (OECS) for continuously monitoring ascorbate in CNS.

2) By using the CNT-based OECS, we compared the dynamic regional changes of extracellular ascorbate level in four different brain regions 1 h after global cerebral ischemia induced by two-vessel occlusion (2-VO). We also compared the change in the level of ascorbate in the different ischemia model (i.e., two-vessel occlusion (2-VO) and left middle cerebral artery occlusion (LMCAO) in striatum.

3) We also demonstrated the validity of the OECS for ascorbate detection as a platform for in vivo evaluation of neuroprotective efficiency of antioxidants by studying the dynamic change of hippocampal ascorbate during the acute period of cerebral ischemia and its responses to intravenous administration of antioxidants including ascorbate and glutathione.

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National Institute on Drug Abuse-Intramural Research Program, NIH, Baltimore, USA

Changes in Brain Oxygen Levels Induced by Heroin and Fentanyl: Evaluation Using High-speed Amperometry in Freely-moving Rats.

While opioid abuse has been a problem for years, increased availability and emergence of more potent synthetic opioids on the market have led to an alarming rise in acute health complications associated with opiate overdose. Respiratory depression followed by brain hypoxia appears to be the most dangerous effect of high-dose opioid use, which could result in a comatose state and death. To explore the effect of opiates on the brain, we employed high-speed amperometry with Pt-based sensors and measured real-time changes in oxygen in the nucleus accumbens of freely moving rats following iv administration of heroin and fentanyl. We observed rapid and potent hypoxia induced by both drugs at human-relevant doses. By measuring oxygen subcutaneously, we confirmed that respiratory depression is the main cause of the brain oxygen decrease. In addition, we observed that opiate-induced hypoxia generalizes to other regions in the brain. Lastly, we employed enzyme-based biosensors to contrast the effect of opiates on oxygen to changes in brain glucose, another critical metabolic substance that also enters the brain from arterial blood. The rapid and potent hypoxic effect we describe in our study highlights the dangerous nature of opiates and the risk these drugs pose to human health.

Supported by the Intramural Research Program of the NIH, NIDA

Symposium 14: Co-transmission in the Nervous System: Unlikely Pairing of Dopamine, GABA, Glutamate, and ACh

Chairs: Nicolas Tritsch, Yan-Feng Zhang

Z. Jimmy Zhou
Departments of Ophthalmology and Visual Science, Cellular and Molecular Physiology, and Neuroscience, Yale University School of Medicine, New Haven, Connecticut, USA

Co-transmission of classic excitatory and inhibitory neurotransmitters in the retina

Amacrine cells are a diverse population of interneurons in the inner retina that participate in complex visual signal processing. They synthesize and release a wide variety of neurotransmitters and neuromodulators and form intricate synaptic circuits with retinal bipolar and ganglion cell types. Many amacrine cell types contain multiple neuroactive substances, thus providing an excellent model system for understanding co-neurotransmission. The two well-known co-release systems in the retina are ACh/GABA and dopamine/GABA co-transmission by cholinergic (starburst) and dopaminergic amacrine cells, respectively. However, it has recently been shown that the amacrine cell type which expresses vGluT3 also co-release two transmitters, namely, glutamate and glycine. The function and circuitry of this novel co-transmission system will be discussed in comparison with the ACh/GABA system in the mammalian retina.

Louis-Eric Trudeau*, G Fortin, C Ducrot, G Osterstock, WM Kouwenhoven, N Giguère
Department of Pharmacology and Physiology, Department of Neurosciences, CNS Research Group, Faculty of Medicine, Université de Montréal, Québec, Canada

On the function and regulation of glutamate co-release by dopamine neurons

Following the cloning and brain mapping of vesicular glutamate transporters (VGLUTs) at the beginning of the millennium, a number of neuronal populations using other primary neurotransmitters were found to also have the capacity to package and release glutamate as a second small molecule neurotransmitter. Subsets of dopamine neurons were thus found to selectively express the vglut2 gene and optogenetic activation of dopaminergic axons confirmed earlier in vitro findings showing that these neurons indeed can release glutamate at synapses. In the present talk, I will first reconsider the axonal domain of dopamine neurons in terms of its structure and presentation of synaptic and non-synaptic axon terminals, a presently unexplained characteristic. I will then present some of the work from my group showing that glutamate release may play a developmental role in dopamine neurons to support their ability to establish release sites in the striatum and perhaps their survival. I will also present results suggesting that release sites for glutamate and dopamine are located at distinct domains along the axons of such neurons. Finally, I will present new results suggesting that the glutamate co-phenotype of dopamine neurons may be plastic in the context of pathology.

N Chuhma, S Mingote, **Stephen Rayport***
Psychiatry – Molecular Therapeutics, Columbia Psychiatry – NYS Psychiatric Institute, New York, USA

Functional connectome mapping of dopamine neuron glutamate cotransmission across the striatum

Dopamine neurons signal via dopamine as well as glutamate and GABA, and differentially target striatal neurons in different striatal regions. Optogenetics has enabled a functional connectomes approach for measurement of dopamine neuron synaptic actions in the striatum directly. In this approach, channel rhodopsin 2 is expressed comprehensively in an identified population of neurons under transgenic control, in this case dopamine neurons, and the incidence, strength and synaptic mediation of connections made by the population of neurons onto identified target neurons determined. This has revealed, in the medial shell of the nucleus accumbens, that dopamine neurons make robust glutamatergic synaptic connections to cholinergic interneurons (ChIs) and weaker connections to spiny projection neurons (SPNs). In the medial dorsal striatum, dopamine neurons make discrete dopaminergic connections to ChIs; in the lateral dorsal striatum, they make slower glutamatergic connections to ChIs and weaker glutamatergic connections to SPNs. Now, in recordings from 100+ neurons of each of the major striatal cell types scattered across the striatum, we are measuring dopamine neuron modulation of firing, synaptic strength, and neurotransmitter mediation, to map dopamine neuron functional connectivity comprehensively.

Yan-Feng Zhang*, SJ Cragg
Department of Physiology, Anatomy and Genetics; Oxford Parkinson's Disease Centre, University of Oxford, UK

Assessing GABA co-transmission from dopamine neurons and its function

Co-transmission of GABA from dopamine transporter (DAT)-positive mesostriatal neurons, alongside the release of dopamine, has been demonstrated in striatum for a few years. This GABA co-release from dopamine neurons seems to have different characteristics from GABA release from classic GABAergic interneurons in the striatum. For example, when recording GABA currents in postsynaptic neurons, the GABA co-release currents show stronger depression when re-activated at short intervals. Also, GABA accumulation in dopamine neurons does not require classic synthesis enzyme GAD, but can involve synthesis requiring aldehyde dehydrogenase 1a1 (ALDH1a1) and reuptake from the extracellular space. However, a systematic comparison of the co-release of GABA and dopamine from dopamine neurons, has been missing.

In the current study, by monitoring extracellular dopamine concentration with fast-scan cyclic voltammetry (FCV), and GABA currents in post-synaptic cells with patch clamp electrophysiology, and using optogenetic stimulation, we revealed that the co-release of dopamine and GABA show some differences. For instance, the decay of the GABA current is faster than dopamine release during a train stimulation, and the recovery of the GABA current is slower. In addition, when exploring the functions for GABA co-release, we found the GABA co-release may act on striatal microcircuits and affect dopamine release in the striatum.

Bradley Roberts*, R Siddorn, NM Doig, SJ Cragg
Centre for Integrative Neuroscience, Department of Physiology, Anatomy and Genetics, University of Oxford, UK; Medical Research Council Brain Network Dynamics Unit, Department of Pharmacology, University of Oxford, UK; Oxford Parkinson's Disease Centre, University of Oxford, UK

Investigating the implications of GABA co-storage in dopamine axons on dopamine transmission

Synaptic transmission between midbrain dopamine (DA) neurons and target neurons in the striatum is essential for action selection and reinforcement. Recent evidence indicates that nigrostriatal DA neurons inhibit striatal projection neurons through the co-release of GABA that acts postsynaptically at GABAA receptors. GABA co-transmission has been shown to be sustained by de novo synthesis catalysed by aldehyde dehydrogenase (ALDH)-1a1 and by GABA uptake via plasmamembrane GABA transporters (GAT), presumably located on DA axons. Given that co-storage of some neurotransmitters has been shown to impact on neurotransmitter levels, we investigated the implications of GABA co-storage on DA transmission, using fast-scan cyclic voltammetry to detect DA at carbon-fibre microelectrodes in acute slices of mouse striatum.

We found that pharmacological inhibition of ALDH did not significantly impact on DA release, but that inhibitors of GAT reduced DA release in the dorsolateral striatum, but not the nucleus accumbens. Direct application of GABA conversely increased DA release, together suggesting that GAT-mediated transport might promote DA release. However, GAT inhibitor effects were reversed by some GABAA inhibitors, suggesting a role for GABAA receptors in the actions of GAT inhibitor drugs, and in turn, in the control of DA release. We discuss further investigations to resolve the regulation of DA release by the GAT and GABA receptors, including immunohistochemical detection of GAT localization on DA axons in the striatum.

Symposium 15: Molecular Monitoring and Modulation during Fear and Anxiety

Chair: Trevor Sharp

Andrew Holmes*, TL Kash, M Penzo, S Silverstein
Laboratory of Behavioral and Genomic Neuroscience, National Institute on Alcohol Abuse and Alcoholism, USA

Monitoring and modulating neural circuits during fear

Posttraumatic stress disorder (PTSD) can result from trauma that is either directly experienced or witnessed, yet most preclinical studies of traumatic fear memory involve direct experience of an innately aversive (unconditioned) stimulus

(US) in association with a neutral (conditioned) stimulus (CS). Various rodent behavioral tasks have been established to assay socially acquired or enhanced fear, but the literature on observational fear remains relatively scant. Here we used a modified version of the standard US-CS Pavlovian fear conditioning paradigm in which an 'observer' mouse witnesses a 'demonstrator' receiving repeated tone-shock CS-US pairings, while hearing the CS but not directly experiencing the US. Employing complimentary behavioral, anatomical and in vivo optogenetics and Ca^{2+} imaging approaches, we sought to define novel mechanisms subserving observational fear. Our findings demonstrate a key contribution of the dmPFC in the formation of observational fear memory and reveal a previously unknown role for ventral hippocampal (vHPC) inputs to the dmPFC pathway in gating this behavior. In addition, we find that specific subpopulations of interneurons in the dmPFC are engaged and causally involved in observational fear. These findings could potentially have therapeutic implications for PTSD and other neuropsychiatric disorders associated with abnormalities in social communication.

T Grund[1], V Grinevich[2], **Inga Neuman[1]***
[1]Department of Behavioral and Molecular Neurobiology, Regensburg Center of Neuroscience, University of Regensburg, Regensburg, Germany; [2]Schaller Research Group on Neuropeptides, German Cancer Research Center, Heidelberg, Germany

Monitoring and chemogenetic manipulation of oxytocin release in distinct brain regions in social behaviour and fear
Our knowledge regarding neuropeptide systems in the brain and their involvement in various socio-emotional behaviours has been significantly boosted by monitoring their release within distinct brain regions in the freely moving rat or mouse using intracerebral microdialysis. One of the best examples of such successful monitoring provide the neuropeptides vasopressin and oxytocin mainly due to the fact that there exist highly sensitive and specific radioimmunoassays making the detection of AVP and OXT in 30-min microdialysates in the pg-range possible.
Thus, AVP and OXT were shown to be released, for example, within hypothalamic supraoptic and paraventricular nuclei, the amygdala, lateral septum, BNST and MPOA during reproductive behaviours such as mating, birth and maternal care, exposure to social stressors, and in response to social interactions such as social investigation or intermale aggression. In addition to these naturally occurring conditions, OXT release can be chemogenetically or optogenetically stimulated within selected brain regions, as indicated by microdialysis, electrophysiologal assessment of target neurons or behavioural consequences, for example during tests for anxiety-related behaviour or in models of contextual or social fear conditioning.

Nicolas Singewald*, TMV Keil, K Kummer, M Kress, K Ebner, SB Sartori
Department of Pharmacology & Toxicology, CMBI, University of Innsbruck, Austria; Department of Physiology and Medical Physics, Medical University of Innsbruck, Austria

Molecular monitoring and modulation of dopamine in aberrant fear processing
Existing treatments of anxiety- and trauma-related disorders show only partial long-term effects. A promising option for improvement is the pharmacological boosting of exposure-based therapy. We show that the dopamine (DA) bioprecursor L-DOPA facilitates fear extinction, the main mechanism of exposure-based therapy, in extinction-intact humans and extinction-deficient 129S1/SvImJ (S1) mice, which was associated with enhanced extinction-induced activity in the vmPFC. S1 mice show blunted neuronal activation in extinction-relevant brain areas such as the vmPFC as compared with extinction-competent c57bl/6 (B6) mice. Further comparing S1 with B6 mice, we studied dynamic changes in extracellular levels of dopamine in the vmPFC during fear extinction using in-vivo microdialysis and, furthermore, revealed differences in downstream DA signaling after L-DOPA using immunohistochemistry. Based on the identified aberrant DA regulation in S1, which was also supported by ex-vivo MEA recordings, we pursued intra-vmPFC dopamine infusion and observed behavioral rescue of the extinction deficit and formation of a persistent fear extinction memory. The receptor(s) mediating this effect are now revealed by microinjections of selective receptor ligands. These results suggest the dopaminergic system as a promising target for the development of pharmacological adjuncts for exposure therapy in extinction-impaired individuals, which are frequent among anxiety disorder patients. Funding FWF-SFB44.

David Bannerman[1]*, J Lima[1], C Barkus[1], T Sharp[2], J Lowry[3], SB McHugh[1]
[1]Department of Experimental Psychology, University of Oxford, UK; [2]Department of Pharmacology, University of Oxford, UK; [3]Department of Chemistry, University of Maynooth, Ireland

In vivo measurement of tissue oxygen and neuronal activity during fear behavior: understanding the role of serotonin in emotion

Translating between human studies and experiments using animal models is notoriously difficult. As well as species differences, we are often comparing across different behavioural tasks and invariably different methodologies. For example, functional imaging studies using the BOLD signal in humans have proved extremely influential for investigating brain function but similar experiments are impossible in freely moving animals. To better enable cross-species comparison, we have pioneered an in vivo amperometric technique that measures changes in brain tissue oxygen concentration at high temporal resolution in behaving rodents. Event-locked, positive-going tissue oxygen responses occur rapidly following neuronal activity and are dependent on neurovascular coupling. Tissue oxygen voltammetry can dissociate between responses in different cortical lamina and between adjacent brain regions during behavioral testing. Recently we have been using this approach in different genetically modified mouse lines, combined with electrophysiological recordings of the local field potential, to investigate the role of serotonin and the serotonin transporter in emotional behavior. Our results may help to resolve controversies from the human imaging literature and can provide important information on the role of serotonin in emotion.

Changwoo Seo[1,2]*, M Jin[1], AK Recknagel[1], E Wang[1], C Boada[1], N Krupa[1], Y-Y. Ho[1], D Bulkin [1,2], MR Warden[1,2]
[1]Department of Neurobiology and Behavior; [2]Cornell Neurotech, Cornell University, Ithaca, NY, USA

Environmental valence modulates dorsal raphe serotonin and GABA neural dynamics

Forebrain serotonin (5-HT) has been associated with an array of behavioral phenotypes that includes behavioral inhibition and learned helplessness. Here, we investigated the role of the dorsal raphe nucleus (DRN), the primary source of 5-HT to the forebrain, in environmental valence. Using fiber photometry, we recorded population activity from DRN 5-HT and GABA neurons while mice were actively behaving in rewarding and aversive environments. We tested these mice in cued approach and avoidance behaviors, in which they were required to cross a chamber either to obtain a reward or avoid a shock. When mice engaged in these visually indistinguishable running behaviors to either obtain a reward or avoid a punishment, DRN GABA neurons continued to be strongly modulated by environmental valence. DRN GABA activity increased during running to avoid the shock and decreased during running to obtain the reward. DRN 5-HT neurons were systematically suppressed during running in both positive and negative environments. Optogenetic manipulation of DRN GABA resulted in context-dependent behavioral changes while DRN 5-HT stimulation resulted in decreased speed in both contexts, consistent with the photometry data. These data support a major role for environmental valence in modulating dorsal raphe neural dynamics during active and inactive behaviors.

Symposium 16: Pre-degenerative Changes in the Dopaminergic System in Parkinson's Disease

Chairs: Sarah Threlfell, Nigel Maidment

K Brimblecombe, B Ryan, D Potgieter, J Larwood, F Serres, T Sharp, N Bengoa-Vergniory, R Wade-Martins, SJ Cragg, **Sarah Threlfell***
Department of Physiology, Anatomy and Genetics, University of Oxford, UK

Pre-degenerative deficits in dopamine transmission in an alpha-synuclein mouse model of Parkinson's disease

In a mouse model of early Parkinson's disease where human α-synuclein is overexpressed (SNCA-OVX) we see an age-dependent loss of dopamine (DA) neurons and emergence of motor dysfunction alongside a reduction in DA neuron firing. Interestingly, prior to neurodegeneration one of the earliest changes in the DA system identified from young adulthood in SNCA-OVX mice is a 30% deficit in DA transmission compared to Snca-/- littermate controls. Moreover, this deficit is region-specific: being most marked in dorsal striatum, a region predominantly innervated by PD-vulnerable

SNc DA neurons. These data suggest early changes in DA synapse function which predates neurodegeneration and may contribute to the subsequent demise of these neurons.
Deficits in evoked extracellular DA ($[DA]_o$) are present throughout SNCA-OVX lifespan (3-22 months) and are not attributable to any deficit in DA content. However, DA uptake is increased in SNCA-OVX and inhibitors of DA uptake, cocaine and GBR 12935, increase $[DA]_o$ to a greater extent in dorsal striatum of SNCA-OVX than Snca-/- controls. Furthermore, DA transporter (DAT) inhibitors eliminate deficits in DA release between SNCA-OVX and Snca-/-. This is not due to increased total DAT levels; however, functional DAT is elevated in SNCA-OVX as revealed via immunofluorescence and radioligand binding studies.

Z Shu[1], HA Lam[1], L Tsan[1], AB West[2], **Nigel Maidment[1]***
[1]Department of Psychiatry and Biobehavioral Sciences, UCLA, USA; [2]Center for Neurodegeneration and Experimental Therapeutics, UAB, USA

Probing dopamine transmission in alpha-synuclein and LRRK2 rodent models of Parkinson's disease using microdialysis and FSCV

It is increasingly recognized that changes in dopamine (DA) transmission precede dopaminergic cell death in Parkinson's disease and may contribute to the heightened sensitivity of DA neurons to degeneration. Earlier work from our laboratory identified elevated striatal extracellular DA in Parkin knockout and human A30P alpha-synuclein over-expressing mice in the absence disruption of DA reuptake, prior to loss of DA terminals or cells, leading us to hypothesize that an increase in tonic DA overflow may be an early event in disease progression. That disruption of DA homeostasis may contribute to the degenerative process is supported by our data showing protection from the neurotoxic effects of alpha-synuclein over-expression by concomitant over-expression of the vesicular monoamine transporter, VMAT2. Our recent work has focused on the influence of the human G2019S LRRK2 mutation on DA transmission, its possible interaction with alpha-synuclein, and the neuroprotective potential of LRRK2 kinase inhibitors. Unlike Parkin KO and A30P alpha-synuclein over-expressing mice, G2019S LRRK2 BAC transgenic rats showed no evidence of elevated tonic extracellular DA by no-net-flux microdialysis, but DA clearance rate was elevated based on this method. FSCV in anesthetized rats revealed facilitation of DA release evoked by short trains of MFB electrical stimulation. This effect was not reversed by acute administration of a LRRK2 kinase inhibitor; the effect of repeated administration is currently under investigation. However, LRRK2 kinase inhibition was protective against the neurotoxic effect of alpha-synuclein preformed fibrils in nigral primary cultures. The apparent pre-degenerative facilitation of dopamine release in the LRRK2 mutant is reminiscent of enhanced dopamine turnover previously identified in asymptomatic human LRRK2 carriers.

Jochen Roeper
Neurophysiology, Goethe University Frankfurt, Germany

Mutant α-synuclein enhances firing frequencies in dopamine substantia nigra neurons by oxidative impairment of A-type potassium channels

Parkinson disease (PD) is an α-synucleinopathy resulting in the preferential loss of highly vulnerable dopamine (DA) substantia nigra (SN) neurons. Mutations (e.g., A53T) in the α-synuclein gene (SNCA) are sufficient to cause PD, but the mechanism of their selective action on vulnerable DA SN neurons is unknown. In a mouse model overexpressing mutant α-synuclein (A53T-SNCA), we identified a SN-selective increase of in vivo firing frequencies in DA midbrain neurons, which was not observed in DA neurons in the ventral tegmental area. The selective and age-dependent gain-of-function phenotype of A53T-SCNA overexpressing DA SN neurons was in part mediated by an increase of their intrinsic pacemaker frequency caused by a redox-dependent impairment of A-type Kv4.3 potassium channels. This selective enhancement of "stressful pacemaking" of DA SN neurons in vivo defines a functional response to mutant α-synuclein that might be useful as a novel biomarker for the "DA system at risk" before the onset of neurodegeneration in PD.

Thomas Barber[1-4]*, L Griffanti[1-4], JC Klein[1-4], M Rolinski[1], F Baig[1], C Ruffmann[1], M Crabbe[1], C Lo[1,2], T Quinnell[5], G Dennis[6], O Bandmann[6,7], G Lennox[8], Z Zaiwalla[9], K Bradley[10], MT Hu[1,2], C Mackay[1,3,4]
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Multimodal neuroimaging in REM sleep behaviour disorder reveals evidence of prodromal neurodegeneration

The degeneration of dopaminergic systems that occurs in Parkinson's disease (PD) begins many years before clinical diagnosis. Neuroprotective interventions may have the greatest potential if applied to this prodromal phase of disease. Patients with idiopathic rapid eye movement sleep behaviour disorder (RBD) have a long-term risk exceeding 80% of developing PD or a closely related condition, and therefore present a unique opportunity to study prodromal disease. During this phase, the degenerative process is thought to ascend through the brainstem to the basal ganglia in a characteristic pattern. We sought to capture evidence of this in patients with RBD using multimodal neuroimaging.

40 patients with polysomnographically-confirmed RBD underwent imaging with DAT SPECT and MRI, including sequences sensitive to neuromelanin. MRI was also performed in healthy control participants.

Using a novel automated MRI segmentation method, RBD patients were found to have reduced substantia nigra (SN) and locus coeruleus (LC) volumes compared to controls. The patterns of abnormality closely matched those seen with DAT SPECT and could differentiate those with clinically abnormal scans from those without. Both SN and LC volumes were inversely correlated with clinical Parkinsonian risk scores.

Neuromelanin-sensitive MRI is a promising biomarker for the detection and stratification of prodromal PD.

Katherine R Brimblecombe*, C Gracie, R Kaestli, SJ Cragg
Department of Physiology, Anatomy & Genetics, University of Oxford, UK

Regulation of L-type calcium channel role in striatal dopamine release: insights for PD

L-type voltage-gated calcium channel (VGCC) function has been identified as a stressor in dopamine (DA) neurons at risk for parkinsonian degeneration. In DA axons, L-type VGCCs regulate DA release in the Parkinson's (PD)-sensitive dorsal striatum, whereas in the PD-resistant ventral striatum, L-type VGCCs do not normally contribute, but can be "un-silenced" by increasing extracellular Ca^{2+} to hyperphysiological levels. But the physiological mechanisms that govern whether L-channels operate to regulate DA release are not well understood.

Here, we have explored candidate endogenous regulators that govern whether or not L-channels operate in DA axons in dorsal versus ventral striatum. We monitored electrically evoked DA release using fast-scan cyclic voltammetry in ex-vivo brain slices from transgenic mouse lines and assessed L-function by testing the effect of isradipine (5 μM). In mice with selective knockdown of calbindin-D28K in DA neurons L-channels were readily recruited in the ventral striatum, revealing that L-type function here is normally limited by calbindin. Furthermore, in α-synuclein-null mice, L-channel function in dorsal striatum was prevented, indicating L-type function here is dependent on α-synuclein.

In summary, L-channel function is regionally regulated in striatal DA axons by α-synuclein and calbindin, which may contribute to their respective toxic and neuroprotective properties in PD.

Posters

BIOSENSORS

1. **J Branigan***, JP Lowry
Neurochemistry Group, Department of Chemistry, Maynooth University, Co. Kildare, Ireland

The development of an electroanalytical biosensor for brain extracellular pyruvate
The objective of this research project is to utilise the enzyme pyruvate oxidase to develop a microelectrochemical biosensor for real-time monitoring of brain extracellular levels of pyruvate. Pyruvate is a chemical of great interest and importance in biochemistry. For, example, pyruvate dehydrogenase complex deficiency (PDCD) is a neurological disorder characterised by the build-up of lactic acid, it results in delayed development in mental abilities and motor skills, and can be potentially fatal. A product of glycolysis it provides energy aerobically via acetyl-CoA and anaerobically in the form of lactate. Publications on the development of pyruvate biosensors have been reported which have involved the use of both pyruvate oxidase and pyruvate dehydrogenase and have generally suffered from sensitivity and selectivity problems rendering them unsuitable for in vivo applications. Here we present preliminary characterisation data on sensitivity (including the effect of cofactor (phosphate) concentrations), linear calibration range and stability, for devices based on development techniques applied to chemically modified carbon and noble metal transducers.

2. **M Clay***, HG Monbouquette
Chemical and Biomolecular Engineering Department, University of California, Los Angeles, Los Angeles, California 90095-1592, USA

A Detailed Model of Electroenzymatic Biosensors Establishes Performance Limitations
A mathematical model of a typical glutamate biosensor consisting of a Pt electrode coated with a permselective polymer film and a layer of crosslinked glutamate oxidase has been constructed in terms of differential material balances on glutamate, H_2O_2 and O_2 in one spatial dimension. Simulations suggest that reducing thicknesses of the permselective polymer and enzyme layers can increase sensitivity ~6-fold and reduce response time ~7-fold, and thereby improve resolution of transient glutamate signals. Oxygen dependence studies with simulated sensors show essentially no reduction in signal at the lowest anticipated O_2 concentrations for expected glutamate concentrations in the brain, and that O_2 transport limitations in vitro are anticipated only at glutamate concentrations in the mM range. The limitations of current biosensors in monitoring glutamate transients is simulated and used to illustrate the need for optimized biosensors to report glutamate signaling accurately on a subsecond timescale. The model has been extended to choline biosensors, and efforts to simulate transport of analytes in brain tissue to sensor sites are underway. This work demonstrates how a detailed model can be used to guide optimization of electroenzymatic sensors and to ensure appropriate interpretation of data gathered using such biosensors.

3. **MM Doran[1]***, KL Baker[1], KW Pierce[1], MD Tricklebank[2], JP Lowry[1]
[1]Neurochemistry Group, Department of Chemistry, Maynooth University, Co. Kildare, Ireland; [2]Department of Neuroimaging Sciences, Institute of Psychiatry, Kings College London, UK

The Development of a D-Amino Acid Oxidase Based Biosensor for the Neurochemical Monitoring of D-Serine
D-Serine is a gliotransmitter that modulates neurotransmission at glutamatergic synapses and has an in-vivo concentration of ~ 6 µM. It is an endogenous co-agonist of glutamatergic N-methyl D-aspartate (NMDA) receptors and has been implicated in disorders such as schizophrenia, cerebral ischemia and Alzheimer's disease. The objective of this research was the development of a novel biosensor for the in-vivo detection of D-serine using Long Term In-Vivo Electrochemistry (LIVE). LIVE requires a microelectrochemical sensor to be implanted in a specific brain region allowing changes in the concentration of the targeted analyte to be detected with sub-second time resolution over extended periods. Experiments were performed in a standard three electrode glass electrochemical cell containing 20 mL phosphate buffer saline, the biosensor, and reference (Saturated Calomel Electrode (SCE)) and auxiliary (Pt wire) electrodes. D-Serine calibrations (0-15 mM) were performed using constant potential amperometry at +700 mV vs. SCE. Several dip-coating procedures were investigated for enzyme immobilisation, including incorporating styrene, glutaraldehyde, bovine serum albumin and polyethylenimine, at various concentrations, in order to develop a simple and reproducible coating method to maximise the sensitivity of the sensor to D-serine. The inclusion of a poly-o-phenylenediamine underlayer conferred excellent permselective characteristics necessary for in-vivo recording.

4. **S Doyle[1]***, KL Baker[1], C Cunningham[2], JP Lowry[1]
[1]*Neurochemistry Group, Department of Chemistry, Maynooth University, Co. Kildare, Ireland;* [2]*School of Biochemistry and Immunology, Trinity College Dublin, Ireland*

Real-time electrochemical monitoring of choline during systemic inflammation in the freely-moving mouse

Cholinergic neurotransmission in the central nervous system plays a crucial role in memory formation and consolidation. Cholinergic neuronal loss, or hypoactivity, is closely associated with cognitive dysfunction, evident in neurodegenerative disorders such as Alzheimer's disease. Moreover, acute systemic inflammation is known to exacerbate working memory deficits and accelerate cognitive decline in patients with prior neurodegenerative pathology. However, the inflammation-induced changes in cholinergic activity are currently unknown.

A novel microelectrode biosensor for the analytical detection of choline, a validated marker of cholinergic activity, has recently been developed and characterised in vivo. Using choline biosensors chronically implanted in freely-moving mice, extracellular choline levels were continuously monitored in both the prefrontal cortex and hippocampus. Scopolamine, a compound known to impair working memory, evoked a transient rise in extracellular choline levels. The loss of cholinergic innervation (p75-saporin lesion in the basal forebrain) abolished the scopolamine-induced choline increase in the hippocampus. Similarly, intraperitoneal injection of bacterial lipopolysaccharide (LPS) evoked a rapid rise in extracellular choline, an effect which was absent in the hippocampus of lesioned mice.

5. **GT Gnahore***, J Branigan, M O'Riordan, JP Lowry
Department of Chemistry, Maynooth University, Co. Kildare, Ireland

The Simultaneous Electrochemical Detection of Serotonin and Dopamine using a Carboxymethyl-β-Cyclodextrin modified Carbon Paste Microelectrode

The present work describes a novel approach for the simultaneous determination of the neurotransmitters serotonin (5-HT) and dopamine (DA), using carbon paste microelectrodes modified with carboxymethyl β-cyclodextrin (CM-β-CD/CPEs). Preliminary in vitro studies indicate that 5-HT and DA, in a solution mixture made of artificial cerebrospinal fluid (aCSF), can be simultaneously oxidised at significantly different potentials in the presence of CM-β-CD, with an anodic peak potential separation of 0.184 V vs. Ag/AgCl. In addition, CM-β-CD/CPEs exhibit up to a 3-fold increase in oxidation current response for both 5-HT and DA, when compared to the unmodified CPE. Future work will involve further characterisation of the developed microsensor with respect to the electrochemical detection of these neurotransmitters in vivo.

6. **JA Holmes***, A West, P Hashemi
Department of Chemistry & Biochemistry, University of South Carolina, USA

Enzyme-free Glutamate Sensing at Ionophore-modified Carbon Fiber Microelectrodes

In recent years there has been a push to expand the boundaries of fast-scan cyclic voltammetry (FSCV) to measure a suite of physiologically relevant signaling molecules in real-time. Our group, among others, has spearheaded this movement with in vivo serotonin and histamine sensing. In this study, our focus shifts towards a fascinating analytical challenge: measuring non-electroactive analytes, specifically glutamate, which is one of the most abundant neurotransmitters. Glutamate is particularly difficult to analyze with voltammetry because it electropolymerizes at biological pH and is structurally similar to many other amino acids found in the extracellular space. We approach this problem from a unique angle by designing a novel ionophore for glutamate binding incorporating Cu(II). We have pioneered Cu(II) FSCV; a method that provides rapid, sensitive, and stable responses to the metal ion. Upon binding to glutamate, the redox peaks associated with Cu(II) in the ionophore shift, offering a quantifiable signal. Here, we describe in detail the development of this novel glutamate sensor and characterize the electrochemical response both in vitro and in vivo. The future of this work is to gain an in depth understanding of glutamate transmission in health and disease comparable to that of other analytes measurable by FSCV.

7. **I Ionescu***, K Allers, R Arban, C Dorner-Ciossek, L Kussmaul
Boehringer Ingelheim Pharma GmbH&Co. KG, CNS Diseases, Biberach, Germany

Glutamate levels measured by glutamate voltammetry in the rat prefrontal cortex after treatment with N-methyl-D-aspartate receptor antagonists

NMDA-R blockers such as ketamine and traxoprodil have been described as potential novel treatment options in the therapy of treatment-resistant depression (TRD). A potential mechanism for antidepressant effects is normalization of the reduced prefrontocortical connectivity in TRD patients. Therefore, we investigated acute changes in glutamate concentration elicited by treatment with different subtypes of NMDA-R blockers in rats in the prefrontal cortex (PFC), using glutamate biosensors.

S-ketamine elicited a significant increase in glutamate in the PFC at 10 mg/kg, a dose which achieves the clinically relevant plasma exposure. The same could be observed after treatment with traxoprodil. Conversely, lanicemine, a clinically inactive NMDA-R blocker, failed to increase glutamate in the PFC at doses up to 30fold of clinically active Cmax. Treatment with (2R,6R)-hydroxynorketamine (HNK) also led to increase in glutamate levels in the PFC.

Here, we show a common effect of behaviorally active doses of the antidepressant-like substances S-ketamine and traxoprodil on glutamate levels in the PFC, whereas lanicemine shows a divergent effect. Therefore, this increase in glutamate levels in the PFC may be an indicator of antidepressant-like effects, indicating its usefulness in proof of concept for the mechanisms of action of candidate compounds with a hypothesized ketamine-like mode of action.

8. **EA Kiyatkin***
National Institute on Drug Abuse-Intramural Research Program, NIH, Baltimore, USA

Beyond Dopamine: Physiological and Drug-induced Changes in Brain Oxygen and Glucose in Freely-moving Rats

Electrochemistry has been intensively used for evaluating rapid fluctuations in dopamine levels in freely moving rats under different behavioural conditions. However, this technology is less popular for monitoring other neuroactive substances. During recent years, we employed high-speed amperometry with glucose and oxygen sensors to examine how these metabolism-related substances fluctuate in the brain's extracellular space under physiological conditions and in response to different drugs of abuse. I will discuss basic features of electrochemical detection of glucose and oxygen in the brain of freely moving rats and present our recent data on physiological fluctuations of these substances induced by natural arousing stimuli. I will also present our recent findings on changes in brain oxygen and glucose induced by cocaine and heroin, two representative drugs of abuse with an opposite behavioural profile. I will demonstrate unique advantages of electrochemical technology for understanding brain entry of oxygen and glucose under physiological conditions and the role of these effects during drug exposure. Lastly, I will discuss the basic mechanisms governing brain entry of glucose and oxygen into the brain and examine relationships between brain entry of these critical metabolic substances and brain metabolic activity.

Supported by NIDA-IRP, NIH

9. L Qi, MS Xiao, **L Li***
School of Chemistry and Molecular Engineering, East China Normal University, Shanghai, China

Poly-cytosine-mediated nanotags for SERS detection

With the aging of the world population, Alzheimer's disease (AD) has become a serious public health issue over the past decades. There is substantial evidence supporting the critical roles of metal ions in the pathogenesis of AD because of their involvement in cell toxicity and epigenetic mechanisms. Among various techniques, surface-enhanced Raman spectroscopy (SERS) provides a powerful analytical tool in biochemical applications. Herein, we have developed poly-cytosine (polyC)-mediated SERS nanotags as a sensor system for rapid, selective, and sensitive detection of Hg^{2+} based on thymidine–Hg–thymidine (T–Hg^{2+}–T) coordination and polyC-mediated Raman activity. The polyC not only provides the anchoring function to induce the formation of intrinsic silver–cytosine coordination but also engineers the Raman-activity of SERS nanotags by mediating its length. As a result, the polyC-mediated SERS nanotags show an excellent response for Hg^{2+} in the concentration range from 0.1 to 1000 nM and good selectivity over other metal ions. This system can be similarly extended to a broad range of biomolecules and metal ions which play important roles in brain chemistry by the design of DNA probe sequences. Given its simple principle and easy operation, this versatile analytical platform could find broad applications in biochemical investigations.

10. **M Malvaez[1]***, C Shieh1, MD Murphy[1], VY Greenfield[1], HG Monbouquette[2], KM Wassum[1]
[1]Department of Psychology, UCLA, USA; [2]Department of Chemical Engineering, UCLA, USA

Amygdala-cortical circuits in reward value encoding and retrieval
The value of an anticipated reward is a key element in the decision to engage in its pursuit. This value is encoded when the reward is experienced in a relevant motivational state. The basolateral amygdala (BLA) is required for this incentive learning process. But whether it also participates in retrieving this information and how it achieves these functions within the broader reward-seeking circuitry is unknown. Using electoenzymatic glutamate biosensors to detect near real-time measurements of BLA glutamate concentration changes, we first found that glutamate is transiently released in the BLA during positive reward value encoding and during value retrieval in subsequent reward seeking. Pharmacological manipulations confirmed the necessity of BLA glutamate receptor activation for both reward value encoding and subsequent value-guided reward seeking. Bidirectional chemogenetic and optogenetic manipulations reveal that activity of orbitofrontal cortex (OFC) projections to the BLA mediate both the encoding and retrieval of a positive reward value change, but there is a double dissociation of the contribution of lateral vs. medial OFC to BLA projections to encoding vs. retrieval, respectively. These data have important implications for the myriad diseases marked by maladaptive reward valuation and decision making.

11. **Z Shu[1]***, X Wen[3], L MacIntyre[1], B Wang[1], HA Lam[1], H Monbouquette[2], P-Y Chiou[3], NT Maidment[1]
[1]Department of Psychiatry and Biobehavioral Sciences, [2]Department of Chemical and Biomolecular Engineering, [3]Department of Mechanical and Aerospace Engineering, UCLA, USA

Integration of optics and microfluidics into MEA biosensors for induction and detection of glutamate release from specific nucleus accumbens afferents in rats
We previously described the development of silicon wafer-based enzyme-linked microelectrode array (MEA) microbiosensors for detection of glutamate, among other analytes, in the rodent brain. The advent of optogenetics permits selective manipulation of genetically identified populations of neurons in the brain. Biosensors provide one means with which to assess the neurochemical consequence of such manipulation. In our own studies of motivated behaviour we seek to manipulate glutamate release from terminals in the nucleus accumbens whose cell bodies originate from distinct anatomical structures, such as regions of the prefrontal cortices, hippocampus and basolateral amygdala. While this can be achieved by remote optogenetic stimulation of these regions, there are advantages to stimulating locally. To this end, we have integrated optical fibers or optical waveguides into our MEA microbiosensors. We have attached 150mm diameter optical fibers to the back of the MEA or imbedded 200nm thick silicon nitride waveguides that emit light, through a grating coupler, from the center of the platinum sensing sites without increasing the dimension of the biosensor. Using these approaches we have detected optically-evoked glutamate release in the nucleus accumbens several weeks after injection of channel rhodopsin-expressing AAV into the above regions, with expression driven by the CAM kinase II promoter. Validation of the recorded signals is aided by incorporation of a thin PDMS microfluidics channel to the back of the MEA to permit local ejection of nanoliter volumes of drug solutions.

12. **E Solis, Jr.***, EA Kiyatkin
National Institute on Drug Abuse-Intramural Research Program, NIH, Baltimore, USA

Changes in Brain Oxygen Levels Induced by Heroin and Fentanyl: Evaluation Using High-speed Amperometry in Freely-moving Rats
While opioid abuse has been a problem for years, increased availability and emergence of more potent synthetic opioids on the market have led to an alarming rise in acute health complications associated with opiate overdose. Respiratory depression followed by brain hypoxia appears to be the most dangerous effect of high-dose opioid use, which could result in a comatose state and death. To explore the effect of opiates on the brain, we employed high-speed amperometry with Pt-based sensors and measured real-time changes in oxygen in the nucleus accumbens of freely moving rats following iv administration of heroin and fentanyl. We observed rapid and potent hypoxia induced by both drugs at human-relevant doses. By measuring oxygen subcutaneously, we confirmed that respiratory depression is the main cause of the brain oxygen decrease. In addition, we observed that opiate-induced hypoxia generalizes to other regions in the brain. Lastly, we employed enzyme-based biosensors to contrast the effect of opiates on oxygen to changes in brain glucose, another critical metabolic substance that also enters the brain from arterial blood. The rapid and potent hypoxic effect we describe in our study highlights the dangerous nature of opiates and the risk these drugs pose to human health.
Supported by the Intramural Research Program of the NIH, NIDA

13. **E Vazquez-Juarez***, M Lindskog
Dept. Neurobiology, Care Sciences and Society, Karolinska Institutet, Sweden

Modulation of Glutamatergic Neurotransmission by Astrocytes

Astrocytes, through glutamate uptake, play an important role in shaping the time course of glutamatergic neurotransmission. High-affinity astrocytic excitatory amino-acid transporters (EAATs) are responsible for the majority of glutamate uptake in a process that involves fast glutamate binding (buffering), followed by transmembrane transport. Different attempts have been made to determine the effects of astrocytic EAATs impairment on the temporal and concentration profiles of extracellular glutamate; however, intrinsic limitations of the techniques used have hindered a comprehensive and detailed picture.

In this work we performed real-time glutamate recordings using enzyme-linked microelectrodes to measure the effect of EAATs inhibition in tonic and evoked glutamate in hippocampal rat brain slices. Tonic glutamate levels in the presence of DL-TBOA a competitive EAAT inhibitor, that prevents glutamate binding, showed a sustained increase; in contrast WAY213613 a non-competitive inhibitor didn't induce a significant increase. The peak amount amount of glutamate evoked by electrical stimulation remained unchanged in the presence DL-TBOA, but a delay in the kinetics of glutamate clearance was observed. Our results add evidence to the role of EAATs in glutamate buffering and provide direct measurements of the effect of EAAT impairment on the temporal and concentration profiles of glutamate.

14. **Y Wang[1]***, Hoda Fathali[1], Jenny Bergman[2], and Ann-Sofie Cans[1]
[1]Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg, Sweden; [2]Department of Chemistry and Molecular Biology, University of Gothenburg, Sweden

A Glutamate Biosensor for Ultra-fast Detection of Glutamate Transients

Glutamate serves as a non-electroactive neurotransmitter in the brain and plays a critical role on learning and memory. To better understand glutamate function, it is important to be able to monitor its rapid transients from neuronal activities which occur on the time scale of milliseconds. Electrochemical methods are predominantly used for probing neurotransmitter release events that occur on the millisecond time scale, but it is limited to detect neurotransmitters that are electroactive, and do not detect neurotransmitters that are non-electroactive.

A novel enzyme-based carbon fiber probe with gold nanoparticles surface modification was recently developed in our group, by limiting the enzyme coating on the sensor surface to a monolayer, these probes display very fast a temporal resolution and respond on the order of milliseconds. Preliminary data using this glutamate sensor in ex-vivo experiments of rodent brain slice show this sensor is fast enough to detection single vesicle glutamate transients on the order of microseconds to millisecond time scale.

15. **P Yu***
Beijing National Laboratory for Molecular Sciences, Key Laboratory of Living Biosystems, Institute of Chemistry, the Chinese Academy of Sciences, Beijing, China

Polyelectrolyte-Modified Micropipette as a New Platform for In Vivo Analysis

Ion current rectification (ICR) is a physical phenomenon that ion current in one direction is greater than that in the other one, which can be attributed to the uneven transport of anions and cations across a nanostructure or a biological channel. ICR obtained at solid state nanopore and nanochannels has recently attracted much attention due to their potential application in fluidic logic circuits (i.e., iontronics),4-6 nanoionics and biosensors. So far, ICR has been observed and investigated in various systems, including nanopipette, conical polymer pores, conical glass pores, SiN nanochannel and protein channels. However, almost all of the reported system was focused on nanoscale since it is difficult to observe the ICR when the diameter of the pores was larger than 10 times debye lengths. Few papers have extended the ICR to micrometer scale by introducing more asymmetric factors. However, there is no report on the observation of ICR at micrometer scale for polyelectrolyte brush modified pore, although many papers have been published by modifying polyelectrolyte brush onto the inner surface of the nanopores.

We interestingly found that micrometer scale ICR can be easily obtained at polyimidazolium brush (PimB) modified pipettes, which provides a new platform for cerebral biological molecular monitoring due to its unique property in easily in operation and relatively robust tip. In this abstract, we would like to introduce our recent research on the in vivo application of polyimidazolium-modified micropipette.

16. **JR Cirrito***, HE Edwards, HL Ridenbark, CM Yuede
Washington University School of Medicine, Department of Neurology, Knight Alzheimer's Disease Research Center, Hope Center for Neurological Disorders, St. Louis, MO, USA

Using micro-immunoelectrodes to study minute-to-minute Aβ peptide clearance kinetics in brain ISF of Alzheimer's mice

The accumulation of amyloid-β peptide (Aβ) in the brain plays a central role in the pathogenesis of Alzheimer's disease (AD). Human studies strongly suggest that a key factor leading to Aβ accumulation is a defect in clearing the peptide from the brain. Several mechanisms involved in Aβ clearance have been identified, and some of these mechanisms may be fast-acting and are unavailable to measure using currently available tools. We have recently developed a novel micro-immunoelectrode (MIE) to detect brain ISF Aβ every 30-60 seconds in living mice, using square wave voltammetry. This new technique provides the temporal resolution necessary to assess very rapid changes in Aβ elimination in living mice. In our design, specificity is achieved by using anti-Aβ antibodies immobilized to the electrode surface. We are using MIEs to determine the rapid kinetics of protein elimination in mice that have suppressed clearance mechanisms. Using the MIEs, we can demonstrate that the elimination half-life of ISF Aβ in vivo is very short (t½ = 23.3 minutes). This tool can be used to assess the fast-acting clearance mechanisms in the brain of living AD transgenic mice.

17. **CM Yuede***; HE Edwards, HL Ridenbark, JR Cirrito
Washington University School of Medicine, Department of Neurology, Knight Alzheimer's Disease Research Center, Hope Center for Neurological Disorders, St. Louis, MO, USA

Studying the temporal relationship between synaptic activity and Ab peptide generation in vivo using microimmunoelectrodes

Alzheimer's disease (AD) is initiated by the progressive accumulation of amyloid-b (Ab) peptide in the brain. Observations in humans show plaques in regions of the brain that display high levels of neuronal activity, referred to as the default mode network (Buckner et al., 2009). Direct modulation of synaptic activity dynamically regulates brain Ab levels in mice; increased synaptic activity increases brain interstitial fluid (ISF) Ab levels and vice versa for suppressed activity. We previously used an in vivo microdialysis technique to demonstrate this relationship between synaptic activity and ISF Ab levels, however, Ab generation likely occurs on a much faster time scale. We recently developed an electrochemical technique using square wave voltammetry to detect Ab in vivo. Ab contains an electroactive tyrosine, which can be detected using a carbon fiber microelectrode. We covalently attached anti-Ab antibodies to the surface of the electrode to provide specificity for Ab over other tyrosines present within the brain. Using the microimmunoelectrode (MIE) we can very rapidly detect changes in brain ISF Ab levels in APP transgenic mice, allowing us to assess fast-acting mechanisms that directly regulate ISF Ab. MIEs provide a novel way to explore mechanisms of this relationship with fine temporal resolution.

ELECTROCHEMISTRY, ELECTROPHYSIOLOGY IN VITRO

18. A Al Ali[1], A Asif-Malik[2], J Canales[3], **A Young[1]***
[1] Department of Neuroscience, Psychology and Behaviour, University of Leicester, UK; [2] Department of Pharmacology, University of Oxford, UK; [3] Department of Psychology, University of Tasmania, Australia

Dopamine D4 mediated attenuation of nucleus accumbens dopamine release measured by fast cyclic voltammetry in rat brain slices in vitro: abolition by phencyclidine pretreatment modelling schizophrenia

The NMDA receptor antagonist, phencyclidine (PCP) induces behavioural changes in rats which mimic schizophrenia symptoms, providing a well validated model of the disease. These effects are reversed by dopamine D4 receptor agonists, including A412997. The current study assessed the effect of PCP pretreatment on A412997-mediated attenuation of electrically-stimulated dopamine release in rat brain slices, using fast cyclic voltammetry (FCV).
Juvenile female Wistar rats were pretreated with PCP (2mg/kg) or saline (1ml/kg) twice daily for 5 days. Following 7 days drug-free, they were humanely killed, brains were removed and consecutive 400µm slices were cut. Dopamine was measured in the background subtracted current signal generated by applying a potential waveform (-0.4 to +1.3 to -0.4 V; 400 V/s) to the electrode. Ten electrical stimulations were applied at 3 minute intervals. After two stimulations, A412997 (2µM) was included in the superfusate for four stimulations, then a further four post-drug stimulations were applied.

In saline pretreated animals, A412997 caused a significant decrease in electrically stimulated dopamine release in NAcS, which was entirely abolished in animals pretreated with PCP. Since PCP pretreatment models schizophrenia, these data suggest that changes in D4 mediated regulation of dopamine release in NAcS may be important in the disease.

19. **KR Brimblecombe***, C Gracie, R Kaestli and SJ Cragg
Department of Physiology, Anatomy & Genetics, University of Oxford, UK

Regulation of L-type channel function in the control of striatal dopamine release: insights for Parkinson's disease
L-type voltage-gated calcium channel (VGCC) function has been identified as a stressor in dopamine (DA) neurons at risk for parkinsonian degeneration. In DA axons, L-type VGCCs regulate DA release in the Parkinson's (PD)-sensitive dorsal striatum, whereas in the PD-resistant ventral striatum, L-type VGCCs do not normally contribute, but can be "un-silenced" by increasing extracellular Ca^{2+} to hyperphysiological levels. But the physiological mechanisms that govern whether L-channels operate to regulate DA release are not well understood.
Here, we have explored candidate endogenous regulators that govern whether or not L-channels operate in DA axons in dorsal versus ventral striatum. We monitored electrically evoked DA release using fast-scan cyclic voltammetry in ex-vivo brain slices from transgenic mouse lines and assessed L-function by testing the effect of isradipine (5 μM). In mice with selective knockdown of calbindin-D28K in DA neurons L-channels were readily recruited in the ventral striatum, revealing that L-type function here is normally limited by calbindin. Furthermore, in α-synuclein-null mice, L-channel function in dorsal striatum was prevented, indicating L-type function here is dependent on α-synuclein.
In summary, L-channel function is regionally regulated in striatal DA axons by α-synuclein and calbindin, which may contribute to their respective toxic and neuroprotective properties in PD.

20. **ZD Brodnik***, RA Espana
Drexel University College of Medicine, Department of Neurobiology and Anatomy, 2900 W Queen Lane, Philadelphia PA, 19129, United States

Cocaine potency at the dopamine transporter is determined by dopamine neuron activation
The reinforcing efficacy of cocaine is largely driven though inhibition of the dopamine transporter, and cocaine potency at the dopamine transporter has been tied to several symptoms of cocaine use disorder. Specifically, high cocaine potency has been tied to excessive motivation to obtain cocaine, and escalation of cocaine taking corresponds with progressive decreases in cocaine potency. While the relationship between cocaine's potency and reinforcing efficacy has become increasingly clear, the physiological determinants of cocaine potency at the dopamine transporter remain unresolved. In these studies we combine chemogenetics with ex vivo fast scan cyclic voltammetry to measure the effects of increases or decreases dopamine neuron activation on cocaine potency. We discovered that changing dopamine neuron activation produced rapid, bidirectional modulation of cocaine potency with increases in dopamine neuron activation driving increases in terminal cocaine potency, and decreases in dopamine neuron activation driving decreases in terminal cocaine potency.

21. **HU Cho***, YM Kang, HJ Shin, CH Park, YB Oh, DP Jang
Department of Biomedical Engineering, Hanyang University, Seoul, Korea

Real-time monitoring of DA release from dopaminergic cell culture using Charge-Balanced Multiple Waveform
It is crucial to monitor dopamine(DA) release in dopaminergic cell culture environment of stem cell study. However, there are lack of methods for real-time monitoring of DA in that condition. Fast scan cyclic voltammetry(FSCV) could be an applicable technique due to high ionic sensitivity, selectivity, and spatial and temporal resolution. Nevertheless, the instability of background currents in conventional FSCV technique limited the monitoring time thus makes it hard to measure slow changes of DA from dopaminergic cell. To overcome this drawback, in this study, we applied charge-balancing multiple fast-scan cyclic voltammetry (CBM-FSCV) combined with dual background subtraction method, which was developed in our previous study. With CBM-FSCV technique we could monitor the endogenously released DA concentration change from ex vivo live dopaminergic cell media over 24 hours with no background drift detected. In addition, it was confirmed for DA cell to be matured after 7-9 day of differentiation. By measuring the long term DA concentration change from ex vivo dopaminergic cell via CBM-FSCV, it is expected that this tool could also be useful method for cell culture related study.

22. **MD Condon***, NJ Platt, SJ Cragg
Department of Physiology, Anatomy and Genetics, Parks Road, University of Oxford, UK

Short-term plasticity of striatal dopamine release is governed by release-independent depression and the dopamine transporter

Dynamic changes in the rate and pattern of action potential generation in midbrain dopaminergic neurons are thought to encode behaviourally-relevant information about salient and/or rewarding stimuli. These neurons project extensively branched axons to the striatum, where the release of dopamine (DA) does not necessarily represent a faithful read-out of presynaptic firing activity. DA signalling will be shaped by presynaptic mechanisms that determine the probability of DA release (Pr) and its short-term plasticity (STP), but these mechanisms are poorly understood. We measured evoked DA release using fast-scan cyclic voltammetry in slices of mouse striatum to explore key candidate mechanisms. DA release is characterised by facilitation at short inter-pulse intervals, and depression at longer intervals. We show that presynaptic STP of DA release is only weakly dependent on release probability, and is governed by release-independent mechanisms related to membrane potential and repolarisation. We demonstrate a role for the dopamine transporter (DAT) as a master regulator of STP, governing both facilitation and depression processes in a region-specific manner. These mechanisms may promote contrast between high- and low-frequency activity, and support diverse roles of DA signalling across striatal regions.

23. **JM Ferdinand***, KZ Peters, AMJ Young
Department of Neuroscience, Psychology & Behaviour, University of Leicester, UK

GABA-B mediated attenuation of accumbal dopamine release is reversed by phencyclidine pretreatment modelling schizophrenia in rat brain slices in vitro.

Dopamine release in nucleus accumbens (NAc) is known to be modulated by GABAergic inputs, both to the cell bodies in ventral tegmental area and to the terminals in NAc. We hypothesised that dysregulation of GABAergic control of dopamine release in the terminals may underlie dopaminergic dysfunctions in schizophrenia. This study aimed to characterise GABAergic modulation of electrically-stimulated dopamine release in brain slices taken from control animals and from animals pre-treated with phencyclidine, in a well validated rat model of schizophrenia. Tissue was stimulated electrically (30 pulses, 60Hz, 500μA) at 3min intervals and dopamine release was measured by fast cyclic voltammetry at a carbon fibre microelectrode positioned in NAc. After four baseline stimulations, drugs were applied in the superfusate for a further four stimulations.Both the GABA-A agonist, muscimol, and the GABA-B agonist, baclofen, caused a significant dose-dependent attenuation of electrically stimulated dopamine release in NAc. In animals pretreated with phencyclidine (2mg/kg, twice daily for 5 days, followed by 7 days drug-free), the attenuation produced by muscimol persisted, but that produced by baclofen was entirely abolished. This abolition after pretreatment with phencyclidine encourages the view that GABA-B (but not GABA-A) mediated control of accumbal dopamine release may be compromised in schizophrenia.

24. **C Gu***, AG Ewing
Department of Chemistry and Molecular Biology, University of Gothenburg, Sweden

Amperometric measurements of the effects of polyunsaturated fatty acids on exocytosis and total vesicle content in PC12 cells

Electrochemical techniques, including single cell amperometry and intracellular vesicle impact electrochemical cytometry (IVIEC), were applied with nano-tip electrodes to examine the effects of alpha-linolenic acid and linoleic acid on the amount of catecholamine released and the vesicular content in a model cell line.

We chose to investigate the fatty acids, which have been implicated in learning deficient hyperactive children previously, as they can alter membrane lipid composition and might subsequently effect on neurotransmission and ultimately change synaptic strength and plasticity. Alpha-linolenic acid and linoleic acid are the two essential fatty acids that cannot be synthesized in the body. Therefore, we chose to study the role of these fatty acids on exocytosis and total vesicle content.

Pheochromocytoma (PC12) cells were used and the results obtained from single cell amperometry and IVIEC showed that both essential fatty acids significantly decreased the monoamine amount being released and the total neurotransmitter amount in the vesicles. Only part of the vesicle load of transmitter is typically released and this fraction did not change upon incubation with the fatty acids. Therefore, these analytical measurements indicate that addition of the two essential fatty acids influence exocytosis by altering the neurotransmitter content.

25. **KM Holleran***, S McCarthy, SR Jones
Department of Physiology & Pharmacology, Wake Forest University Baptist Health, Winston Salem, NC, USA

Alterations in nucleus accumbens dopamine dynamics and negative-affective like behaviors are driven by stress and ethanol exposure alone and in combination
Comorbidity between alcohol use disorder (AUD) and disorders of negative affect – such post-traumatic stress disorder (PTSD) – is exceedingly high, and hypodopaminergia is thought to underlie the anhedonia common to these conditions. Stress is believed to imbue vulnerability to both AUD and disorders of negative affect. We used two models of stress exposure in C57Bl/6J mice, repeated forced swim stress (FSS) and mouse single prolonged stress (mSPS), either alone or in conjunction with chronic intermittent ethanol (CIE) exposure. We found that CIE drives negative affect-like behaviors during withdrawal via the marble burying task and novelty-suppressed feeding task. Exposure to mSPS, a recent model of PTSD, increased anxiety-like behavior in a novel open field. We examined dopamine (DA) dynamics in the nucleus accumbens using voltammetry after CIE and/or stress. CIE reduced stimulated DA release up to 4 weeks after withdrawal and increased reuptake rate in early withdrawal. Stress exposure increased stimulated release and reuptake rate. Finally, both stress and CIE exposure augmented the dopamine-inhibiting effect of kappa opioid receptor (KOR) agonist stimulation. Taken together, these data indicate that EtOH and/or stress exposure exert powerful long-lasting alterations to affective states that may be driven through neural adaptations of the nucleus accumbens dopamine system.

26. **YM Kang***, HJ Shin, CH Park, HU Cho, YB Oh, DP Jang
Department of Biomedical Engineering, Hanyang University, Seoul, Korea

A User-friendly toolbox for long-term neurochemical measurement using Multi-waveform fast-scan cyclic voltammetry
FSCV(Fast scan cyclic voltammetry) has been used as a technique that estimates the changing concentration of neurotransmitters in the brain. Conventional FSCV is unsuitable for long-term measurement because the background signal is unstable. Recently, we developed charge-balancing multiple waveform and dual subtraction technique to minimize temporal variation in its background current. However, there is no toolbox or system for general researchers to use multiple waveform FSCV acquisition and processing. In this research, we suggested general purpose toolbox to perform not only conventional FSCV but also multiple waveform FSCV. The software consists of two part, real-time data acquisition software and post data analysis software. The data acquisition software controls the data acquisition(DAQ) devices to acquire the voltammogram from single waveform and multiple waveform. The post data analysis software contains various analysis functions for multiple waveform FSCV. It is expected that this toolbox could be useful tool for general researchers who wants to use multiple waveform FSCV and to get a long-term measure while minimizing the effect of background drift.

27. **AN Karkhanis***, JL Weiner, SR Jones
Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC, USA

Adolescent social isolation augments kappa opioid receptor function in the nucleus accumbens and basolateral amygdala of rats
Adverse experiences during adolescence increase alcohol use disorder vulnerability during adulthood in humans. Rats exposed to adolescent social isolation (aSI) show greater ethanol intake in adulthood compared to group housed (aGH) controls. Acute stress elevates dynorphin levels, a kappa opioid receptor (KOR) ligand, and KOR activation inhibits dopamine release in the NAc and the BLA, two interconnected regions integral in stress and reward-seeking behavior. Baseline dopamine levels were lower in the NAc and BLA of aSI rats and were reversed following KOR-inhibition. Ethanol-induced dopamine elevations were greater in NAc and BLA of aSI rats. KOR-inhibition augmented ethanol-induced dopamine responses in the NAc but attenuated them in the BLA of aSI rats. The inhibitory effects of KOR-activation on dopamine release were enhanced in the NAc of aSI rats suggesting that chronic stress augments KOR function. KOR-overexpression in dopamine neurons augmented ethanol intake in aGH rats. KOR-inhibition differences may explain ethanol's effects on behaviors related to specific brain regions, e.g., ethanol-induced augmented dopamine in the NAc increases reinforcement, whereas augmented dopamine in the BLA may reduce heightened anxiety levels. Therefore, KOR inhibition in the NAc attenuates reinforcing value and reduces anxiety decreasing effects of ethanol in addition to decreasing overall anxiety.

28. **A Larsson***, S Majdi, A Oleinick, C Amatore, A Ewing
Department of Chemistry and Molecular Biology, University of Gothenburg, Sweden; Départment de Chimie, CNRS, France; Department of Chemistry and Chemical Engineering, Chalmers, Sweden

Determination of total octopamine content in a living neuron using amperometry and mathematical modelling
Previously, our groups have quantified the released amount of octopamine from a Drosophila larva neuromuscular junction (NMJ). Interestingly, several of the detected events occurred through so-called complex events, where the fusion pore seems to flicker during "partial" release. This flickering is one potential mechanism neurons have to adjust the strength of their signalling and would thus be of value to investigate with relation to synaptic plasticity. To address this, the total amount of catecholamine inside each vesicle has to be quantified.
By combining a fluorescently labelled larva and the previously described nanotip carbon fiber electrodes, we were able to position the electrode inside the bouton at the NMJ and detect vesicular content in situ. As the vesicles adsorb and burst on the electrode, we are able to quantify octopamine from the resulting current as it is oxidized. The other approach includes using earlier data from release events at the NMJ as basis for mathematical modelling. This method has been used before to estimate both fusion pore size and vesicular content. These two approaches result in distributions of similar shapes and values verifying that we are truly measuring content in these synaptic vesicles. However, the octopamine content is considerably larger when compared to release events, indicating that only a very small fraction is released during exocytosis at the NMJ.

29. **E Lopes***, SJ Cragg
Department of Physiology, Anatomy and Genetics, University of Oxford, UK

The role of GABA receptors in regulating striatal dopamine release
Dopamine (DA) released from midbrain afferents plays a crucial role in the normal functioning of the striatum. The majority of intrinsic striatal neurons release GABA, and nigrostriatal DA neurons also co-release GABA, but whether striatal GABA directly impacts on striatal DA release remains unclear. This study aims to address whether GABAA and GABAB receptors modulate DA release in dorsal striatum, measured using fast-scan cyclic voltammetry in mouse striatum.
Activation of GABAB receptors with the agonist baclofen modestly reduced DA release evoked electrically or by optogenetic activation of DA axons. We assessed further whether these effects were mediated directly on DA axons or involved cholinergic interneurons which strongly regulate DA via striatal nicotinic receptors (nAChRs). In the presence of a nAChR antagonist, activation of GABAB receptors continued to reduce DA release, corroborating a direct effect on DA axons. These effects were attributable to GABAB receptors as they were prevented by the GABAB antagonist saclofen.
Activation of GABAA receptors by the agonist muscimol, or inhibition by the GABAA channel blocker picrotoxin had mixed effects on DA release that appeared to vary with stimulus intensity or type. Emerging results will be discussed.
These results suggest that striatal GABA can directly modulate DA release through at least GABAB receptors.

30. **CH Park[1]***, JK Kim[1], YB Oh[2], YM Kang[1], HJ Shin[1], HW Cho[1], DP Jang[1]
[1]Department of Biomedical Engineering, Hanyang University, Seoul, Korea; [2]Department of Neurologic Surgery, Mayo Clinic, Rochester, MN, USA

Large amplitude fast square wave cyclic voltammetry for enhancing selectivity of neurotransmitters
Although fast-scan cyclic voltammetry has widely been used in neuroscience, it has still limited selectivity in catecholamines differentiation, such as dopamine or norepinephrine. In this study, we proposed a new type of voltammetric technique, which we called large amplitude Fast Square Wave Cyclic Voltammetry (FSWCV), for improving a selectivity of neurotransmitters in the brain. Large amplitude FSWCV consists of a large amplitude periodic square pulse signal (>0.3V) superimposed on a staircase waveform. Using the periodic characteristics of an acquired FSWCV voltammogram, it was rearranged into two dimisional (2D) voltammogram image instead of conventional 1D voltammogram in FSCV. It contains 2D redox patterns as well as electron transfer rate patterns. In results, the 2D voltammograms from various analytes such as dopamine, 5HT, or pH showed their unique features using this technique. By combining principal component regression, we showed a significant improvement in the differentiation of dopamine, epinephrine and norepinephrine in their mixture compared to FSCV($P<0.05$). In addition, it was evaluated for the detection of dopamine in the striatum in vivo. In conclusion, large amplitude FSWCV would be one of promising tools in neuroscience research area.

31. **BM Roberts***, R Siddorn, NM Doig, SJ Cragg
Centre for Integrative Neuroscience, Department of Physiology, Anatomy and Genetics, University of Oxford, UK; Medical Research Council Brain Network Dynamics Unit, Department of Pharmacology, University of Oxford, UK; Oxford Parkinson's Disease Centre, University of Oxford, UK

Investigating the implications of GABA co-storage in dopamine axons on dopamine transmission
Synaptic transmission between midbrain dopamine (DA) neurons and target neurons in the striatum is essential for action selection and reinforcement. Recent evidence indicates that nigrostriatal DA neurons inhibit striatal projection neurons through the co-release of GABA that acts postsynaptically at GABAA receptors. GABA co-transmission has been shown to be sustained by de novo synthesis catalysed by aldehyde dehydrogenase (ALDH)-1a1 and by GABA uptake via plasmamembrane GABA transporters (GAT), presumably located on DA axons. Given that co-storage of some neurotransmitters has been shown to impact on neurotransmitter levels, we investigated the implications of GABA co-storage on DA transmission, using fast-scan cyclic voltammetry to detect DA at carbon-fibre microelectrodes in acute slices of mouse striatum.
We found that pharmacological inhibition of ALDH did not significantly impact on DA release, but that inhibitors of GAT reduced DA release in the dorsolateral striatum, but not the nucleus accumbens. Direct application of GABA conversely increased DA release, together suggesting that GAT-mediated transport might promote DA release. However, GAT inhibitor effects were reversed by some GABAA inhibitors, suggesting a role for GABAA receptors in the actions of GAT inhibitor drugs, and in turn, in the control of DA release. We discuss further investigations to resolve the regulation of DA release by the GAT and GABA receptors, including immunohistochemical detection of GAT localization on DA axons in the striatum.

32. E Yavas, **BM O'Connor***, AMJ Young
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Cholinergic mechanisms modulating nucleus accumbens dopamine release: a link to schizophrenia?
Dopamine release in nucleus accumbens (NAc) is important in processing motivational and attentional information, and it has been suggested that dysregulation of dopamine release may underlie positive symptoms of schizophrenia. There is now substantial evidence that release is critically influenced by modulatory systems acting on dopamine neurone terminals in NAc, through a number of neurotransmitter systems, including acetylcholine. Further, it has been suggested that disruption of cholinergic control of accumbal dopamine release, either by a direct action on dopamine terminals, or via other intermediate neurones (e.g. glutamatergic, GABAergic) may contribute to behavioural deficits seen in schizophrenia. This study therefore aimed to investigate the effect of phencyclidine pretreatment, modelling schizophrenia, on both nicotinic and muscarinic cholinergic control of electrically stimulated accumbal dopamine release, mimicking phasic activation, in rat brain slices in vitro, using fast cyclic voltammetry at a carbon fibre microelectrode.
Results confirmed that both nicotinic and muscarinic mechanisms modulate dopamine release in NAc, in a dose dependant manner. Moreover this regulation was disrupted in slices taken from animals pretreated with phencyclidine (2mg/kg, twice daily for 5 days, followed by 7 days drug-free), encouraging the view that dysregulation of cholinergic control of accumbal dopamine release may be important in schizophrenia.

33. **YF Zhang[1,2]***, John NJ Reynolds[2] and Stephanie J Cragg[1]
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Pauses in cholinergic interneuron activity are driven by excitatory input and delayed rectification, with dopamine modulation
Cholinergic interneurons (ChIs) of the striatum pause their firing in response to salient stimuli and to conditioned stimuli after learning. Several different mechanisms for pause generation have been proposed but a unifying basis has not previously emerged. Here, using in vivo and ex vivo recordings in rat and mouse brain and a computational model, we show that ChI pauses are driven by withdrawal of excitatory inputs to striatum and result from intrinsic biophysical properties of ChIs in concert with local neuromodulation. A delayed-rectifier potassium current (IKr) enables ChIs to report changes in excitatory input, to pause on input recession and to scale pauses with strength of excitatory input, in keeping with the pause acquisition during learning. We show also how pauses can be modulated by dopamine through

inhibition of ChIs directly, but primarily by strengthening excitatory inputs. These findings provide a unifying basis for pauses in striatal ChIs.

ELECTROCHEMISTRY – IN VIVO DOPAMINE

34. **EA Budygin***
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Exploring phasic changes in striatal dopamine release under the effect of negative stimuli
The perception and consequences of pleasurable and negative stimuli are different, and the underlying substrates mediating these opposing phenomena are unclear. To address the question of how dopamine (DA) neurotransmission within the striatum encodes negative stimuli, we evaluated DA dynamics in response to aversive stimuli using voltammetry. Surprisingly, we found that the neurochemical and anatomical substrates responsible for the perception and processing of pleasurable, rewarding stimuli were activated by tail pinch. Thus, tail pinch triggered transient increases in extracellular DA concentration in the nucleus accumbens (NAc) of freely moving rats. These transients did not differ from those triggered by rewarding stimuli. To avoid the influence of other possible stimuli, striatal DA responses were explored in anesthetized rats. We found that pinch-evoked DA release in the dorsal striatum and NAc core was time locked to the duration of the stimulus. However, DA was released in the NAc shell only when tail pinch was terminated. Furthermore, it was revealed that unpainful tail touch could also induce DA transients but only if it was previously coupled with the painful stimulus. Our data suggest the existence of some overlap in the neurochemistry of perception and processing of negative and positive stimuli within striatum.

35. **LM Burgeno[1,2,3]***, NL Murray[1], RD Farero[1], JS Steger[1,2], ME Soden[1,2], I Willuhn[1], LS Zweifel[1,2], PEM Phillips[1,2]
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Diametric Changes in Striatal Dopamine Release Underlie Drug-Taking and Drug-Seeking Behaviors
Though altered dopamine transmission is implicated in most contemporary theories of addiction, the timing, context, and directionality of these changes remain a matter of debate. While some studies demonstrate dopamine in the nucleus accumbens core (NAcc) plays an important role in producing drug satiety, others suggest NAcc dopamine mediates craving and promotes drug seeking. How might drug-cue elicited dopamine transmission in the NAcc serve both as a satiety signal and to produce 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, presented unexpectedly during abstinence, signal possible drug availability nearby and promote drug-seeking. For NAcc dopamine to both decrease drug-taking and increase drug-seeking, we hypothesize there must be a divergence in dopamine evoked by drug-paired cues when presented in drug-taking vs. -seeking contexts. To test this hypothesis, we used fast-scan cyclic voltammetry to measure changes in drug-cue elicited dopamine over time in both of these contexts. Indeed, we find that while cue-elicited dopamine transmission significantly decreases during drug-taking ($p<0.05$, as we previously published), dopamine responses to the same cue increase during drug-seeking ($p<0.01$).

36. **ES Carlson[1]***, SG Sandberg[1], TM Locke[1], PEM Phillips[1,2], and LS Zweifel[1,2]
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Genetic Dissection of Catecholaminergic Innervation of the Cognitive Cerebellum
Studies in humans and non-human primates have identified a region of the dentate nucleus of the cerebellum (DCN), or lateral nucleus in rodents (LCN), which is activated during performance of cognitive tasks and is implicated in psychiatric illnesses. We have shown that the dopamine D1 receptor marks a population of LCN neurons with similar spatial distribution and regulates performance on tasks related to navigation and working memory, and connects with other parts of the brain classically involved in these functions. However, virtually nothing is known about the basic anatomical and functional organization of the LCN. We hypothesized that the locus coeruleus (LC) is the principal source of catecholamine release in LCN, and that catecholamines are required for cerebellar enhancement of cognitive tasks. Mapping experiments in mice revealed projections of the LC to DCN, and stimulation of LC resulted in catecholamine release in the LCN measured with fast scan cyclic voltammetry. When tyrosine hydroxylase expression is genetically inactivated in input projections to the LCN, abnormal performance on discrimination of fear predictive cues, working

memory, and impulsive behaviors is observed. Finally, we have preliminary data showing fluctuation of catecholaminergic release in voltammetric recordings from LCN in freely moving mice.

37. **AL Collins[1]***, TJ Aitken[1], V Greenfield[1], SB Ostlund[2], KM Wassum[1,3]
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Nucleus accumbens acetylcholine modulates cue-evoked dopamine to regulate cue-motivated reward-seeking
Reward-predictive stimuli provide a major source of motivation for reward-seeking actions. Considerable evidence has implicated the nucleus accumbens core (NAc), and dopamine signaling therein, in the expression of a cue's motivational value. Interestingly, the striatal cholinergic system is capable of terminally modulating dopamine release and influencing local circuit dynamics within the NAc. This led us to hypothesize that the striatal cholinergic system may provide a regulatory mechanism over dopamine release and appetitive behaviours. However, little is known about how striatal acetylcholine regulates cue-related dopamine release to mediate cue-motivated reward seeking. Therefore, we assessed the causal role of NAc cholinergic interneuron activity in mediating the ability of a reward-paired cue to invigorate reward-seeking actions by manipulating the activity of cholinergic interneurons during a Pavlovian-to-instrumental transfer (PIT) test. We then assessed the hypothesis that activity at NAc acetylcholine receptors terminally modulates dopamine release to mediate cue-motivated reward seeking using fast-scan cyclic voltammetry. We further monitored fluctuations in NAc acetylcholine release during the PIT task using choline biosensors. Collectively, these results elucidate the role of the NAc cholinergic system in acting as a supressatory gate on cue-evoked dopamine signaling to regulate the excitatory influence of a reward-paired cue over reward seeking.

38. **Dan P Covey[1]***, E Hernandez[2], CE Bass[3], S Patel[4], JF Cheer[1,5]
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Endocannabinoid synthesis by dopamine neurons controls cue-directed motivation
Mounting clinical and preclinical work demonstrates that endocannabinoid (eCB) signaling controls adaptive and pathological forms of reward seeking, but the mechanisms remain elusive. 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, how eCBs shape DA function and reward pursuit is not known. To elucidate how the eCB 2-arachidonoylglycerol (2-AG) controls reinforcement and DA function, we selectively deleted the 2-AG synthesizing enzyme diacylglycerol lipase α (DGLα) from VTA DA neurons, and measured real-time DA dynamics in the NAc using fast-scan cyclic voltammetry during sucrose reinforcement. Conditional, targeted deletion of DGLα 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. Deficits in operant responding and DA dynamics further increased as effortful costs (i.e., lever presses) escalated. Alternatively, DGLα deletion has no effect when reward receipt is not reliant on cue processing and response cost is minimal. These findings demonstrate that 2-AG mobilization from VTA DA neurons controls how DA neurons encode environmental stimuli and direct motivation.

39. **A Huber***, L Oikonomidis, EM Tunbridge, ME Walton
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Genetically-encoded differences in cortical dopamine affect phasic dopamine release in nucleus accumbens and modulate the effect of cue salience on associative learning
Animals use cues to predict the occurrence of reward in a process dependent on striatal dopamine. Whilst much is known about striatal dopamine and reward prediction errors, it is unclear whether other factors influencing learning are mediated by dopamine. Some cues are more attention-grabbing or salient than others, which can affect learning rates. We hypothesised that cortical dopamine might mediate this relationship between salience and learning.
To investigate this hypothesis, we used a transgenic mouse model mimicking a polymorphism found in the human catechol-O-methyltransferase gene (COMT Val158Met). COMT plays an important role in breakdown of dopamine in cortex, and its activity is reduced in Met-allele carriers.

COMT-Met mice and wild type controls were trained on a Pavlovian conditioning paradigm where auditory cues (low–salience 3kHz tone or high–salience white noise) preceded reward delivery. We found cue identity had a greater impact on learning in COMT-Met mice than controls.
We used fast-scan cyclic voltammetry to track phasic dopamine release in nucleus accumbens core (NAcC) as COMT-Met and WT mice learned Pavlovian associations. COMT genotype, and therefore cortical dopamine, influenced NAcC dopamine release evoked by cues, but not rewards.
These data suggest cortical-striatal dopamine circuitry may mediate salience effects in associative learning.

40. **C Korn[1,2]***, T Akam[2], A Huber[1,2], KHR Jensen[1,2], C Vagnoni[1,2], EM Tunbridge[1], ME Walton[2]
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Distinct roles for DAT and COMT in regulating dopamine transients and reward-guided decision making
Mechanisms for regulation of dopamine transmission are critical to its effects on behaviour and vary by region. Recycling via the dopamine transporter (DAT) predominates in striatum, while degradation by catechol-O-methyltransferase (COMT) predominates in cortex. However, questions remain about whether and how each mechanism affects fast fluctuations in dopamine transmission in these regions and influences behaviour.
Fast scan cyclic voltammetry recordings of evoked dopamine release in anaesthetized mice revealed that DAT blockade enhanced dopamine transients in nucleus accumbens but did not affect transients in frontal cortex. Unexpectedly, COMT inhibition had no effect on transients in either region. In contrast, both amphetamine and a noradrenaline transporter blocker increased evoked release in cortex, indicating that mechanisms other than DAT and COMT regulate cortical transmission at sub-second timescales.
We assessed the roles of DAT and COMT in behaviour using a multi-step decision making task that requires mice to monitor changes in both optimal response strategy and reward probabilities. DAT blockade selectively decreased value learning rate and impaired performance following changes in reward probability but not in response strategy. DAT blockade also quickened responding and increased trial rate. By contrast, COMT inhibition selectively improved adaptation to changes in reward probability without affecting other performance measures.

41. **AR Kosheleff[1,2]***, L Tsan[1], F Gomez-Pinilla[3], SB Ostlund[4], NP Murphy[1], NT Maidment[1]
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High-fructose diet increases reward seeking and dopamine signalling in rats
Consumption of palatable foods, like many abused drugs, increases dopamine (DA) signalling in mesolimbic regions of the brain. With repetition, such increases in DA transmission become associated with proximate cues that can trigger further reward seeking, which may be a factor in overeating. Diets high in refined carbohydrates impair insulin signalling in the periphery (e.g., Type II Diabetes), and recent evidence suggests insulin resistance can also occur in the brain. Since the DA transporter (DAT) is positively regulated by insulin, we hypothesized that neuronal insulin resistance due to chronic fructose exposure would persistently downregulate DAT activity, reducing extracellular DA clearance, resulting in increased DA signalling that manifests as hypersensitivity to food-paired cues. We found that a high-fructose diet significantly impaired insulin signalling in the ventral midbrain and rendered rats hypersensitive to the motivational impact of environmental cues in a Pavlovian-to-instrumental transfer (PIT) test. Using in-vivo fast-scan cyclic voltammetry (FSCV) in anesthetized rats, we found DA reuptake to be impaired in the ventral striatum of fructose-exposed animals. These behavioural and neurochemical effects were prevented by administration of the diabetes-treatment drug, pioglitazone. Finally, using FSCV in awake, behaving rats, we found DA release events associated with environmental cues and food-seeking during a PIT test to be enhanced in fructose-exposed animals. These data highlight a potential role insulin dysregulation in mediating diet-induced hypersensitivity to environmental cues, an action that may be due to compromised DAT function.

42. H Saoud, T Pouvreau, D De Beus, S Eybrard, **A Louilot***
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Early functional blockade of the ventral subiculum enhances ketamine-induced dopaminergic responses in the core part of the nucleus accumbens in adult rats
A striatal dopaminergic (DA) dysregulation in schizophrenia is commonly acknowledged and may be dependent of a subiculo-striatal disconnection involving glutamatergic NMDA receptors. The present study was designed to investigate, in adult rats, the effects of the non-competitive NMDA receptor antagonist ketamine on DA responses in core part of

the nucleus accumbens (Nacc), following an early functional blockade of the left ventral subiculum (SUB). Functional blockade of the left SUB was carried out by local tetrodotoxin (TTX) microinjection at postnatal day 8 (PND8), i.e. a critical time of the neurodevelopmental period. DA variations were recorded using in vivo voltammetry in freely moving adult rats (11 weeks). The following results were obtained: 1) A dose effect was observed for the two microinjected groups (PBS and TTX) at PND8; 2) DA increases in the core part of the Nacc in adult animals after the administration of ketamine were more elevated in TTX microinjected animals than in PBS microinjected animals. These data suggest that animals microinjected with TTX in the left SUB at PND8 present a more important reactivity to ketamine than control animals. In conclusion, these findings may provide new insights regarding the involvement of NMDA glutamatergic receptors in the pathophysiology of schizophrenia.

43. **M Niello[1]***, K Jäntsch[1], HH Sitte[1], D Walther[2] MH Baumann[2]
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In vitro and in vivo characterization of 1-phenyl-2-(pyrrolidin-1-yl)pentan-1-one (α-PVP) enantiomers

1-Phenyl-2-(pyrrolidin-1-yl)pentan-1-one (α-PVP) is a synthetic analog of the naturally-occurring stimulant cathinone, a main psychoactive compound in the Khat plant. 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 racemic α-PVP show it acts as a potent inhibitor of DAT and NET (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. Here, we examined the activity of α-PVP enantiomers on monoamines transporters in vitro using uptake inhibition assays, and in vivo using fast scan cyclic voltammetry (FSCV) in rats. Our findings demonstrate (S)-PVP is the bioactive enantiomer in the racemic mixture.

44. **MC Panayi[1]#*, T Jahans-Price[1]#***, T Boerner[1]#, A Huber[1,2], ME Walton[1], DM Bannerman[1]
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Glutamatergic dysfunction leads to a hyper-dopaminergic phenotype: Linking dopamine to aberrant salience

Aberrant salience, the inappropriately persistent and high levels of attention paid to stimuli, is the dominant mechanism of psychosis in schizophrenia and thought to be mediated by elevated levels of dopamine. Recent large scale GWAS meta-analyses have established a significant association between schizophrenia and the Gria1 locus coding for the GluA1 subunit of the AMPA glutamate receptor. GluA1-KO mice have previously been studied in relation to schizophrenia but, notably, striatal whole tissue levels of dopamine and its metabolites appear normal in these animals. However, it has yet to be determined whether they exhibit dynamic, behaviour-dependent and stimulus-specific changes in dopamine.

To test this we recorded dopamine signals in the nucleus accumbens using fast-scan cyclic voltammetry in behaving wild-type and GluA1-KO mice. Neutral light stimuli evoked prominent dopamine signals in all mice. Crucially, these failed to habituate in GluA1-KO mice, resulting in a behaviourally-relevant, hyper-dopaminergic phenotype in these animals. In addition, dopamine responses to unsignalled rewards were also significantly enhanced in the knockout mice. However, preliminary data suggests that evoked dopamine in anaesthetised GluA1-KO mice is no different to WTs. Thus, we provide evidence for behaviourally-relevant hyper-dopaminergic responses in a genetically modified mouse model of glutamatergic dysfunction relevant to schizophrenia.

45. **AG Schindler[1]***, JS Meabon[1,6], Marcella K[5], Pagulayan KF[1],6, Li G[1,6], Sikkema C[5], Wilkinson CW[1,5], E Peskind[1,6], JJ Clark[1,4], DG Cook[2,3,5]
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Persistent behavioral dysfunction following blast exposure in mice and combat veterans: Potential role for dysfunctional phasic dopamine release

Mild traumatic brain injury from blast exposure (blast-mTBI) has been called the "signature injury" of OIF/OEF/OND. Of significant concern is potential for post-concussive symptoms and behavioral dysfunction (e.g. irritability, impulsiveness,

risk taking, aggression, substance abuse, PTSD, depression), but diagnoses and treatment options are limited. The mesolimbic dopamine system plays a role in motivation, reward processing and decision making, but perturbations are implicated in dysfunctions similar in nature to those seen following blast-mTBI. Here we used a battlefield-relevant mouse model to investigate the effects of blast on the mesolimbic dopamine system as a potential underlying neurochemical mechanism of blast-induced behavioral dysfunction. In mice, blast exposure increased sensation/novelty seeking and irritability, and engendered conditioned place aversion, behaviors closely associated with risk of substance abuse in humans, which is of increasing concern for veterans with blast-related mTBI. Increased PTSD, risk-taking behaviors and irritability symptoms were reported by blast-mTBI Veterans. In accordance with these behavioral outcomes, blast increased evoked phasic dopamine release in the nucleus accumbens core. Taken together these findings suggest that blast-related mTBI provokes persistent changes to the dopaminergic system. Such changes may mediate aspects of the complex behavioral dysfunction reported in blast-exposed Veterans and provide potential new therapeutic targets.

46. **Helen N. Schwerdt***, Min Jung Kim, Elizabeth Zhang, Satoko Amemori, Tomoko Yoshida, Robert Langer, Michael J. Cima, Ann M. Graybiel
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Multi-site monitoring of subsecond dopamine neurochemical activity in rodents
Dopamine-related neurochemicals are implicated in numerous debilitating disorders and govern fundamental behaviors. These molecules operate in site-specific circuits in the brain, and measuring their stratified functions is important in identifying disease etiology. We have developed multichannel carbon fiber arrays that allow voltammetric sampling of dopamine and other electroactive compounds from multiple sites (up to 16) at spatial intervals of 250 micrometers. We manufactured fast scan cyclic voltammetry (FSCV) instrumentation that provides the capacity to record subsecond chemical fluctuations synchronously from 16 channels. We tested the arrayed system by recording from up to 16 sites across the dorsal striatum of anesthetised rats and controllably evoked striatal dopamine by medial forebrain bundle stimulation and administration of drugs. Acute recording experiments demonstrated highly heterogeneous patterns of striatal dopamine release that were differentially modulated in relation to stimulation locations and drug administration. We further characterized protocols to mark focally recorded sites to evaluate potential differential effects on specific cell types by post-perfusion histochemistry. Work is underway to increase channel count, and to classify spatially the dynamics of striatal dopamine signals and their relation to striatal circuit organization. This work was supported by NIH R01 EB016101, R01 MH060379, and F32 NS093897.

47. **Z Shu[1]***, HA Lam[1], AB West[2], NT Maidment[1]
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Monitoring dopamine dynamics in a G2019S LRRK2 rat model of Parkinson's disease
Mutations in Leucine-rich repeat kinase 2 (LRRK2) are responsible for a large number of familial Parkinson's disease (PD) cases and variation in the LRRK2 genetic locus is a risk factor for sporadic PD. The most common mutation, G2019S, results in increased kinase activity. Consequently, LRRK2 kinase inhibitors are being aggressively developed as a potential treatment strategy, but much remains to be learned about the role of LRRK2 in neuronal function and the mechanism by which LRRK2 overactivity impacts neuronal survival. However, mounting evidence supports a role for LRRK2 in vesicle cycling and dopamine homeostasis. We are using fast-scan-cyclic voltammetry (FSC) in a human G2019S LRRK2 BAC transgenic rat to probe the influence of the mutation on dopamine (DA) dynamics prior to degeneration of DA cells or terminals. FSCV coupled with carbon fiber microelectrodes implanted in the dorsal striatum of anesthetized rats revealed augmented DA responses evoked by electrical stimulation of the medial forebrain bundle, preferentially during 6-pulse ultrashort stimulations. Kinetic analysis of the DA responses showed that the G2019S mutation did not change the apparent Vmax or KM of DA uptake but rather increased DA release at the beginning of the stimulation. Pharmacological manipulation of the DA transporter (DAT) with nomifensine confirmed that impairment of DAT is not likely to account for the elevation in evoked DA signals. However, normalization of responses in the present of the D2 autoreceptor, raclopride, suggests an impairment in D2-mediated autoinhibition in the mutant.

48. **W van Elzelingen***, WE Bastet, JM Matos, I Willuhn
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Evolution of phasic dopamine release in the striatum during reward seeking
With repeated performance, goal-directed behavior that reliably results in desired outcomes is often automated and executed habitually. Some evidence suggests that this switch in behavioral strategy may be reflected in a shift in dopamine efflux from the ventromedial striatum (VMS) to the dorsolateral striatum (DLS). Thus, we used fast-scan cyclic voltammetry in freely-moving rats to investigate how dopamine signaling in both the VMS and DLS evolves during the development of habitual reward seeking.
Food-restricted Long-Evans rats were operant conditioned to seek food pellets on a chained seeking-taking reinforcement schedule in an operant box. Pressing the seeking lever provided access to a taking lever under a variable interval 60 s schedule. Subsequent responding on the taking lever under a fixed ratio 1 schedule resulted in the delivery of a food reward. After stable responding was reached, reward devaluation sessions were performed to test whether animals executed food seeking habitually. Voltammetry measurements were conducted repeatedly throughout training to detect changes in dopamine release during the development of habitual responding. Preliminary results indicate the presence of task-related phasic dopamine signals in both the VMS and DLS throughout the training, and thus seemingly independent of behavioral strategy or automaticity.

ELECTROCHEMISTRY – IN VIVO OTHER MOLECULES

49. **SN Berger***, P Hashemi
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How Does Pesticide Exposure Effect Monoamine Transmission?
Pesticides are frequently used in the industrial and consumer sectors for the control of pests on crops and greenery. Many pesticides function by disrupting key neurochemical messengers in pests. This mechanism of action raises the question of the impact pesticide exposure has on the human brain. Despite the well-established methods to ascertain the toxicity of compounds, toxicity to the brain is much more difficult to define. In this work, we apply fast-scan cyclic voltammetry to measurements of serotonin and histamine in mice that have been exposed to chlorpyrifos, a hallmark organophosphate pesticide. We explored the effects on neurochemistry of different doses, exposure routes and exposure times of this chemical. The general trend is that chlorpyrifos reduced serotonin transmission in exposed mice. Exposure to organophosphates such as chlorpyrifos has previously been thought to induce neuroinflammation. We hypothesize histamine levels in the brain to increase during neuroinflammation and since we recently observed that histamine negatively modulates serotonin, we test the notion that chlorpyrifos' effects on serotonin are via histamine receptors. This study will allow a greater understanding of the neurotoxicological effects of pesticide exposures have on the human brain.

50. **MA Booth[1]***, MM Stevens[1,2], MG Boutelle[1]
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New technologies for the delivery and monitoring of chemicals in the brain
In vivo voltammetric electrochemical sensors and biosensors have the great advantage of high temporal resolution when compared to microdialysis, however, have the disadvantage of uncertainly of calibration and lack the capability to deliver drugs locally. We are examining a number of approaches to address these problems including the development of novel hybrid devices combining the best aspects of both measurement techniques. This poster will describe the design and construction of novel tools aimed at neurochemical monitoring. We will discuss the detection methodologies of the devices and examine their ability to deliver and monitor both exogenous and endogenous chemicals within the brain.

51. **Anna Marie Buchanan[1,2]***, Parastoo Hashemi[1]
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In Vivo Fast Scan Cyclic Voltammetry Analysis of Serotonin in a Neurodegenerative Disease Model
Over the past decade, there has been a significant increase in the prevalence of neurodegenerative diseases. While the mechanisms into the cognitive and motor functions of these diseases have been studied extensively, the early symptoms of anxiety and depression that accompany neurodegenerative diseases, which are highly debilitating, are not well understood. One challenge to understanding these symptoms is the lack of methods available to study the underlying neurotransmitters and ions in vivo. To better understand this problem, fast scan cyclic voltammetry (FSCV) was used to measure serotonin and some ions in vivo in a variety of different neurodegeneration mouse models. We describe the development and application of FSCV to measuring ions such as Cu(II) in vivo. Additionally, we discuss data observing significant differences in the chemistry between normal and neurodegeneration models. Future studies aim to better understand the non-motor symptoms of neurodegenerative diseases.

52. **Al Gerth***, MF Roitman
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Fast-scan cyclic voltammetry (FSCV) reveals evoked phasic fluctuations of norepinephrine in the Paraventricular Nucleus of the Hypothalamus (PVN)
The PVN is a critical node in directing neural, hormonal and behavioral responses to perturbations in homeostasis. It expresses receptors for catecholamines including those for norepinephrine (NE), dopamine (DA) and epinephrine (E). Here, we sought to characterize catecholamine release in the PVN using FSCV – an electrochemical technique that can sample the release of electroactive neurotransmitters with good analyte specificity. FSCV was performed in the PVN of anesthetized rats while the ventral noradrenergic bundle was stimulated once every 5 minutes. Representative cyclic voltammagrams from peak current changes were regressed against those obtained during post-recording calibration where the electrode was exposed separately to 1uM NE, 1uM DA and 1uM epinephrine (E). Cyclic voltammograms obtained in vivo compared favorably to NE and DA but not E. NE and DA are indistinguishable using FSCV. Thus, in vivo experiments also employed selective pharmacological agents. NE (desipramine, yohimbine) but not DA (GBR-12909, raclopride) selective drugs modulated evoked signals in the PVN whereas the opposite was found during recordings in the striatum and in response to medial forebrain bundle stimulation. Thus, NE is the principal catecholamine detected with FSCV in the PVN. Ongoing work seeks to determine how psychostimulant exposure modulates the NE response.

53. **M Hersey[1,2]***, SA Samaranayake[2], A Abdalla[2], LP Reagan[1,3], P Hashemi[2]
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Histaminergic Modulation of Serotonin During Chronic and Acute Neuroinflammation
Neuroinflammation closely correlates with changes to the central nervous system (CNS) and plays a role in the pathology of many neurological diseases, including depression. Our lab has strong interests in probing the neurochemical underpinnings of depression in the context of serotonin and histamine. We are interested in serotonin because of the long hypothesized notion that serotonin signaling is impaired during depression. We are interested in histamine because of this messenger's well-established role in peripheral inflammation and new data from our lab showing that histamine release inhibits serotonin signaling. In this work, fast-scan cyclic voltammetry (FSCV) is used to simultaneously measure histamine and serotonin, in the posterior hypothalamus of the mouse in acute (peripheral injection of lipopolysaccharide) and chronic (high fat diet (45 kcal % fat)) neuroinflammation models. Endocrine (for inflammation) and behavioral (for depression) analysis will be correlated with neurochemical measurements. Our preliminary results allow us to hypothesize that inflammation corresponds to increased histamine release, thus increased inhibition of serotonin. We also find that the capacity of selective serotonin reuptake inhibitors, like escitalopram, to increase extracellular serotonin is reduced during both inflammation models. Our data highlights the important role that histamine plays in modulating serotonin during inflammation and thus depression.

54. **Hochner B[1]***, Stern-Mentch N[1,2], Nesher N[1,2], Shomrat T[1,2], Turchetti-Maia AL[1]
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Long-term potentiation (LTP) expression and maintenance in the octopus vertical lobe is mediated by long-term elevation in nitric oxide (NO) concentration

The octopus vertical lobe (VL), a brain area that controls the sophisticated learning of this invertebrate, demonstrates a robust activity-independent NMDA-independent LTP. We show here that the presynaptic expression of LTP involves activation of nitric oxide synthase (NOS) and that nitric oxide (NO)-dependent reactivation of NOS functions as a 'molecular switch' mediating the very long, protein synthesis-independent, LTP maintenance (>10h). While NADPH-diaphorase histochemistry supports the presence of NOS in the VL, we could not find any indication for the involvement of NO-dependent cGMP cascade in LTP. Additionally, NO-donors and NO-scavengers had no effect. These negative results suggest the possible involvement of processes that function at high NO concentration (e.g., s-nitrosylation).Therefore, we measured NO concentration amperometrically and found that induction of LTP is accompanied by a long-term increase in the amperometric signal that corresponded with the oxidation potential of NO (750 mV). The increase was to around µM ranges; much higher than that found for the activation of the cGMP cascade. We therefore hypothesize that a process such as s-nitrosylation could serve as an effective mediator of a local retrograde message for ensuring specificity in presynaptic LTP.
Support: ISF, BSF and the National Inst. For Psychobiology (to TM and NN)

55. **MA Johnson***, KJ Garcia, T Schneider, MJ Sofis, SM Lemley, SV Kaplan and DP Jarmolowicz
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Mechanisms of chemotherapy-induced impairments in executive function

Chemotherapy-related cognitive impairment (CTRC, 'chemobrain'), an impairment of executive function caused by administration of adjuvant chemotherapy for non-central nervous system malignancies, is expected to affect one third of the 19 million cancers survivors in the next decade. Although recent studies have implicated a variety of causes, such as the formation of reactive oxygen species and inflammation, the specific mechanisms that underlie cognitive impairment are still not clearly defined. Our group has found previously that dopamine and serotonin release are impaired in rats treated with carboplatin. In this work, we will report on neurochemical methods combined with enhanced behavioral methods designed to identify how specific components of executive function are affected. Moreover, we have previously treated rats with KU-32, a heat-shock protein inhibitor, and found that this drug prevents the onset of cognitive impairment in rats. We will discuss potential neurochemical and molecular mechanisms that underlie this beneficial effect.

56. **CA Lee***, SK Smith, SE Calhoun, CJ Meunier, GS McCarty, LA Sombers
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Monitoring Real-Time Opioid Peptide Fluctuations with Multiple Scan Rate Voltammetry Reveals a Neuromodulatory Role for Met-Enkephalin in the Rat Striatum

Opioid signalling in dopaminergic circuits is critically implicated in natural and drug reward-seeking behavior, as well as in motor control. However, the precise modulatory action of opioids remains ambiguous. Although several methods exist for monitoring dopamine in reward-related brain nuclei, few tools are available for selectively monitoring dynamic fluctuations of opioid neuropeptides in striatum. We utilized a novel fast-scan voltammetric approach to monitor dynamic concentrations of endogenous tyrosine-containing opioid peptides, such as methionine-enkephalin (M-ENK), in rat striatum. This approach utilizes two scan rates and an amperometric hold in every voltammetric sweep to afford reproducible, sensitive, and selective measurements. Importantly, our electrochemical approach enables simultaneous detection of multiple chemical species in each scan, including dopamine. Thus, we have simultaneously measured rapid fluctuations of dopamine and M-ENK in the dorsal striatum of an adult, male rat, and investigated chemical dynamics during consumption of unexpected, palatable food reward. The signals were pharmacologically verified with infusion of protease inhibitors near the recording electrode surface, which increased extracellular M-ENK concentrations in a predicable manner. The preliminary data suggest that M-ENK may augment dopaminergic tone in the dorsal striatum. Collectively, these measurements elucidate a modulatory role for dynamic, striatal M-ENK fluctuations in dopamine-associated behaviors and pathologies

57. **R Robke[1]***, I Willuhn[2], and P Hashemi[1]
[1]Chemistry and Biochemistry, University of South Carolina, USA; [2]Department of Psychiatry, Academic Medical Center, University of Amsterdam, NL

Innovating Serotonin Measurements in Freely-Moving Animals: Compulsive Model
Obsessive-compulsive disorder (ODC) is prevalent in 1-3% of the world's population and is comorbid with other neuro-psychiatric disorders such as addiction, anxiety, and depression. Currently, the most prescribed treatment for OCD is serotonin-selective reuptake inhibitors (SSRIs), which acutely act on the serotonin transporter to increase extracellular levels of serotonin. In this work, we aim to develop fast-scan cyclic voltammetry to monitor serotonin release in the prefrontal cortex of freely-moving rats performing scheduled-induced polydipsia (SIP). We utilize Sprague Dawley rats, that display a stable SIP response after 15 days, with high drinkers displaying nearly 4 times as severe compulsive symptoms as low drinkers. Individual differences in serotonin neurotransmission will be investigated between the high and low drinkers. Acute SSRI administration allows us to test the mechanistic features of the therapeutic effects of this agent for OCD. This collaborative work will a) provide the community with novel technological advancements in freely moving serotonin voltammetry and b) shed light on the roles that serotonin plays in OCD.

58. **Y Wang***, B. Jill Venton
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Characterization of spontaneous, transient adenosine release in various mouse brain regions
Adenosine is a neuroprotective endogenous agent that modulates important physiological process in the central nervous system. Spontaneous, transient adenosine has been recently identified as a rapid mode of signaling that lasts about three seconds. However, whether this transient adenosine release is different between brain regions and the extent to which adenosine is released as adenosine itself or as a breakdown product of ATP is not fully known. Here, spontaneous, transient adenosine release in the prefrontal cortex, hippocampus and caudate putamen of anesthetized mice were characterized using fast-scan cyclic voltammetry. The average concentration of adenosine in the prefrontal cortex (0.267 ± 0.053 μM) was significantly higher than in the hippocampus (0.093 ± 0.012 μM) and caudate putamen (0.095 ± 0.001 μM). However, the numbers of adenosine release were not significantly different between three brain regions. In addition, spontaneous adenosine was measured in CD73 knockout mice, which lack ecto-5' nucleotidases. CD73 mice have significantly fewer adenosine transients than the control but there was no significant change of adenosine concentration, indicating that only some of the spontaneous, transient adenosine were formed from the breakdown of ATP. These findings provide better understanding of the brain region variations and the mechanisms of transient adenosine release, which will be helpful to design better strategies to control adenosine and activate its neuroprotective effects for future disease related treatments.

59. **A West***, P Hashemi
Department of Chemistry & Biochemistry, University of South Carolina, USA

Evaluating the Fundamental Serotonin Chemistry of the Prefrontal Cortex in the Context of Autism Spectrum Disorder
Autism spectrum disorder (ASD) is a collection of developmental disorders with growing prevalence. The pathophysiology of ASD is not yet fully understood, hindering effective prevention and treatment options. Specifically, a universal underlying chemical mechanism is lacking. We believe that serotonin dysfunction can be identified as a common neurochemical mechanistic feature of this disorder, however current techniques do not provide a complete representation of the serotonin system as they are only capable of measuring basal levels at a temporal resolution that does not reflect fast synaptic changes. Here we describe the application of fast-scan cyclic voltammetry, which operates on a neurotransmission temporal resolution, to examine serotonin release and reuptake in the medial prefrontal cortex of genetic ASD models. These models allow us to establish a chemical phenotype accompanying stereotypical ASD behaviours in mice. The results demonstrate a significant difference in the serotonin chemistry between ASD models and controls. Identifying this chemical phenotype will allow us to redefine the serotonin chemistry within ASD.

FLUORESCENCE-BASED IMAGING

60. **D A Beccano-Kelly***, Y Mousba, J Vowles, S Cowley, R Wade-Martins
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Intracellular calcium signalling pathway is disturbed in iPSC derived neurons from patients with genetic autosomal dominant forms of Parkinson's disease

Current mammalian models of Parkinson's disease (PD) do not fully recapitulate the pathophysiology, which may contribute to the lack of current therapeutics for the disease. As such, we have begun to use human induced pluripotent stem cell (hiPSC) derived DAns in an effort to define the ontology of the disease, in a model more relevant to human neurodegeneration. Previous data showed ER (important in calcium signalling) stress in iPSC derived DAns from PD patients.

Using ratiometric calcium assays and western blotting technique, we demonstrate that these neurons have a significantly reduced amount of calcium released from intracellular stores upon pharmacological stimulation. This deficit seems attributable to reduced signalling proteins, phospholipase D1 and calcium-independent phospholipase A2. Furthermore, this effect is positively correlated with exogenous increases of the PD associated protein, α-synuclein, and can be phenocopied by incubation of IPSC derived DAns with said protein.

Calcium is key to processes such as ATP generation and neurotransmitter release; these are an order of magnitude higher in DAns whose processes are long and unmyelinated. As a result, energy demand and calcium homeostasis are delicately balanced in DAns, and thus disruption of calcium signalling may confer dopamine neuron vulnerability in PD.

61. **H Bischof[1]***, E Eroglu[1], B Gottschalk[1,2], F Hellal3, M Rehberg[3], M Waldeck-Weiermair[1], N Plesnila[3], WF Graier[1,2], R Malli[1,2]
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Novel genetically encoded fluorescent probes enable real-time detection of potassium in vitro and in vivo

We have recently developed Förster resonance energy transfer (FRET)- based potassium sensitive probes, allowing the real-time visualization of intra- and extracellular K^+ dynamics. These probes, referred to as GEPIIs (genetically encoded potassium ion indicators), consist of a bacteria-derived K^+ binding protein (Kbp) sandwiched by a cyan and a yellow fluorescent protein variant. Upon K^+ binding to these chimeras, FRET signals increase dramatically, yielding a high signal to noise ratio. Recombinant GEPIIs can be used to quantify K^+ levels in diverse bodily fluids in a fast and convenient manner. Moreover, GEPIIs are suitable to monitor cell viability and cell growth with high spatial and temporal resolution. In addition, purified GEPIIs can be applied in vivo for the visualization of extracellular K^+ dynamics in tissues of living animals. Expression of GEPIIs in cells allows hitherto unfeasible real-time monitoring of subcellular K^+ dynamics. Our data highlight organelle specific K^+ fluxes in response to cell depolarization. Such subcellular K^+ signals might have multiple implications in cell physiology and pathology. We highlight that GEPIIs are suitable for diverse K^+ assays and open new avenues for live-cell K^+ imaging.

62. **X Cui***
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Phosphors in Substituted Rhodamine with Bridge-Caging Strategy for in vivo Enzyme Imaging

Dynamic monitoring the activity of enzyme in vivo is of critical importance for understanding their activities in both physiological and pathologic processes in brain[1]. Rhodamine is a versatile fluorogenic framework for bioimaging. The substitution of bridging atom in rhodamine by phosphorus is one of the most efficient way to achieve near-infrared (NIR) bioimaging[2, 3]. We synthesized a series of phosphorus-substituted NIR rhodamine, where phosphors not only act as the spectral tuner, but also provide a specific "caging" site with unique fluorogenic mechanism. Caging group ensures the colorless, closed form of spiro-rings in with high stability in wide pH range ($5 < pH < 10$), while de-caging process after specific stimulus results ring-opened form with strong fluorescence. Excellent hydrophilicity of the fluorophore has ensured lossless fluorescence efficiency in the absence of organic solvent. The developed system with general fluorogenic strategy has been justified as a powerful platform for the imaging of enzymes in brain in vivo.

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63. **Chiacchierini G***, Peters KZ, Naneix F, Snoeren EMS, McCutcheon JE
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Restriction of dietary protein alters preference for protein and associated neural activity in ventral tegmental area
Of the three macronutrients, dietary protein seems to be the most tightly regulated. Despite this, there has been little research into how the brain encodes internal states relating to restriction of protein and how this affects preference and motivation for protein-containing foods. We have compared protein-restricted rats (maintained on 5% protein diet) to control, non-restricted rats (on 20% protein control diet) and found that protein-restricted rats show an increased preference for protein-containing solutions (4% casein), relative to carbohydrate solutions (4% maltodextrin). To examine the underlying neural mechanisms, we expressed a calcium indicator, GCaMP6s, in ventral tegmental area (VTA) neurons and used vivo fibre photometry to measure calcium fluctuations as a proxy for neural activity associated with preference for protein. By controlling access to protein- and carbohydrate-containing solutions using retractable sippers we were able to examine patterns of activity related to consumption of each macronutrient. We found that neural activity in VTA was increased during bouts of licking for both protein-containing and carbohydrate-containing solutions. However, in protein-restricted rats, this activity was greater when consuming protein than carbohydrate. Thus, this elevation was related to the magnitude of protein preference. Ongoing experiments are exploring how internal state is communicated to VTA neurons to be converted into behavioural output.

64. **TM Hsu***, VR Konanur, MF Roitman
Department of Psychology, University of Illinois at Chicago, USA

Thirst and the hormone Angiotensin II recruit VTA dopamine signalling to water consumption
Hunger and hunger-related hormones (e.g. ghrelin) can strongly modulate mesolimbic dopamine signalling and reward encoding. Here we investigated whether changes in body fluid homeostasis, specifically thirst, can impact the activity of VTA dopamine neurons. To examine VTA dopamine neuronal activity in awake behaving animals, we utilized in vivo fiber photometry in rats expressing Cre-recombinase under the control of a tyrosine hydroxylase promoter (TH-Cre+) together with a Cre-dependent AAV packaged with a Ca^{2+} indicator, GCaMP6f targeting the VTA (AAV1-Syn-Flex-GCaMP6f). In water-deprived animals, our results first demonstrated substantial changes to VTA dopamine neuron Ca^{2+} transient characteristics, particularly increases in amplitude and signal duration, in response to water consumption compared to water-sated animals. Next, to investigate whether central hormone modulation can mimic thirst evoked VTA dopaminergic responses, we delivered the dispogenic hormone, Angiotensin II (AngII) to the lateral ventricles of water-sated TH-Cre+ rats. Similar to water-deprived animals, we found that central administration of AngII changes VTA dopamine neuron Ca^{2+} transient characteristics in response to water consumption in comparison to vehicle treated animals. Overall, our data suggests that thirst can powerfully influence VTA dopamine activity and highlights the importance of physiological state in mediating mesolimbic dopamine function.

65. **KD Jovanoski***, G Das, S Waddell
Centre for Neural Circuits and Behaviour, University of Oxford, UK

Investigating state-dependent nutrient learning and memory in Drosophila
Drosophila dopaminergic neurons provide teaching signals to synapses of the fruit fly mushroom body during feeding. Anatomically distinct subsets of dopaminergic neurons appear to reinforce water and separate properties of sugars: sweet taste reinforces short-term memories while nutrient input is required for long-term memories. Here we explored dopaminergic reinforcement at the physiological level using two-photon in vivo calcium imaging during repetitive droplet feeding.
When starved flies first feed, dopaminergic neurons projecting to the horizontal tip of the mushroom body show a calcium response that reflects salience and whose magnitude increases with sweetness. This initial feeding calcium response cannot be increased by adding caloric content to sweet-only sugars. Calcium responses in these neurons to subsequent feeds of sweet-only sugars may reflect reward prediction error because they gradually diminish. Interestingly, calcium responses to repeated feeds of the sweet and nutritious sugar sucrose remain unchanged until

the fly reaches satiety. Moreover, we were surprised to find that in dehydrated flies, these dopaminergic neurons show calcium responses of similar magnitude to water and sugar-water, suggesting that their signals are strongly influenced by deprivation state.

66. **VR Konanur***, and MF Roitman
Department of Psychology, University of Illinois at Chicago, USA

Using in vivo Fiber Photometry to Further Understand Mechanisms of Amphetamine Action
In brain slices, amphetamine (AMPH) decreases electrically-evoked, phasic dopamine release, which is thought to be due to vesicular depletion and reverse transport. In behaving rats, however, AMPH increases the frequency of phasic dopamine release events (thought to be driven by burst firing at dopamine cell bodies). Thus, we hypothesized that AMPH would enhance the neuronal activity of dopamine neurons in behaving rats. To explore this possibility, we used in vivo fiber photometry in rats expressing a genetically-encoded calcium indicator in dopamine neurons to serve as a proxy for neuronal activity at the cell body. To validate this approach, we first monitored calcium transients in response to stimuli known to modulate both dopamine neuronal activity and phasic dopamine release. A sucrose-paired cue evoked a time-locked rise in calcium; and spontaneous calcium transients were modulated by dopamine autoreceptor pharmacology. Finally, we found that AMPH (1 mg/kg, i.p.) decreased the frequency of spontaneous calcium transients. This last finding suggests that AMPH-induced phasic dopamine release may be decoupled from neuronal activity at the cell body. Ongoing experiments will compare AMPH responses to those of other drugs and determine if dopamine terminals are more excitable following AMPH administration.

67. **J Lezmy[1]***, M Lipinsky[1], Y Khrapunsky[2], E Patrich[1], L Shalom[1], A Peretz[1], I Fleidervish[2], B Attali[1]
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M-current inhibition rapidly induces a unique CK2-dependent plasticity of the axon initial segment
Alterations in synaptic input, persisting for hours to days, elicit homeostatic plastic changes in the axon initial segment (AIS), which is pivotal for spike generation. Here we triggered in hippocampal pyramidal neurons of both primary cultures and slices a unique form of AIS plasticity by selectively targeting M-type K^+ channels, which prominently localize to the AIS and are essential for tuning neuronal excitability. While acute M-current inhibition via cholinergic activation or direct channel block made neurons more excitable, minutes to hours of sustained M-current depression resulted in a gradual reduction in intrinsic excitability. Dual soma–axon patch-clamp recordings combined with axonal Na^+ imaging and immunocytochemistry revealed that these compensatory alterations were associated with a distal shift of the spike trigger zone and distal relocation of FGF14, Na^+ and Kv7 channels but not Ankyrin G. The concomitant distal redistribution of FGF14 together with Nav and Kv7 segments along the AIS suggests that these channels relocate as a structural and functional unit. These fast homeostatic changes were independent of L-type Ca^{2+} channel activity but were contingent on the crucial AIS protein, protein kinase CK2. Thus, alterations in M-channel activity rapidly trigger unique AIS plasticity to stabilize network excitability.

68. **KZ Peters***, AMJ Young, JE McCutcheon
Department of Neuroscience, Psychology & Behaviour, University of Leicester, UK

Distracting stimuli evoke responses in ventral tegmental area neurons during ongoing saccharin consumption
Attention disruptions and distractibility are implicated in multiple disorders. The ventral tegmental area (VTA) sends projections to nucleus accumbens and cortical areas involved in guiding attention. Given the dual role of dopamine signals in both driving ongoing behaviours (e.g. feeding) and monitoring salient environmental stimuli, understanding the interaction between these functions is crucial. We have investigated modulation of VTA neuronal activity during distraction from ongoing feeding. We developed a distraction assay exploiting self-paced licking in rats trained to lick for saccharin. Once licking across session was stable a distraction test was performed where three consecutive licks within 1 second triggered a random distractor (e.g. light/tone). Rats were judged not distracted on trials where they continued to lick, ignoring the distractor. Conversely, pauses in licking indicated distraction. We expressed GCaMP6s in VTA neurons and used fibre photometry to assess calcium fluctuations during this task as a proxy for neuronal activity. Although activity increases were evoked by both initiation of consumption and onset of distractors, the latter predominated. Thus, peak responses to distractors were significantly higher than to licking alone. We are currently

determining the specific neuronal subpopulations involved and whether these activity patterns are disrupted in models of schizophrenia.

69. **RA Saylor[1]***, S Lumbreras[2], AM Buchanan[1], J Raymond[2], T Lau[2], and P Hashemi[1]
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Investigation of Serotonin Transporter Dynamics in Response to SSRI Administration
Selective serotonin reuptake inhibitors (SSRIs), prescribed for depression, are thought to act by blocking serotonin reuptake via inhibition of serotonin transporters (SERTs), though many questions remain as to the precise mechanism of action, dose regimen, and time course surrounding this treatment. Through in vivo fast-scan cyclic voltammetry after acute SSRI administration, we determined that evoked serotonin release and reuptake responds in a dose-dependent, but not linear, manner. Unexpectedly, we also observed a faster reuptake of serotonin at select time points and SSRI doses. This lead us to hypothesize that the surface expression of SERT must increase dynamically in response to SSRI administration, compensating for increased extracellular serotonin levels. To test this theory, we studied SERT function and density in vitro in serotonergic neuronal stem cells through imaging techniques. Our in vitro results show both an increased SERT function and surface density in active neurons as quickly as 5 minutes after SSRI administration. Taken together, our in vivo and in vitro results demonstrate a rapid, potent increase in SERT function and surface expression after acute SSRI administration. Our results have the potential to revolutionize SSRI therapy, as this increase in reuptake has never before been considered.

70. **C Seo[1,2]***, M Jin[1], AK Recknagel[1], E Wang[1], C Boada[1], N Krupa1, Y-Y Ho[1], D Bulkin[1,2], MR Warden[1,2]
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Environmental valence modulates dorsal raphe serotonin and GABA neural dynamics
Forebrain serotonin (5-HT) has been associated with an array of behavioral phenotypes that includes behavioral inhibition and learned helplessness. Here, we investigated the role of the dorsal raphe nucleus (DRN), the primary source of 5-HT to the forebrain, in environmental valence. Using fiber photometry, we recorded population activity from DRN 5-HT and GABA neurons while mice were actively behaving in rewarding and aversive environments. We tested these mice in cued approach and avoidance behaviors, in which they were required to cross a chamber either to obtain a reward or avoid a shock. When mice engaged in these visually indistinguishable running behaviors to either obtain a reward or avoid a punishment, DRN GABA neurons continued to be strongly modulated by environmental valence. DRN GABA activity increased during running to avoid the shock and decreased during running to obtain the reward. DRN 5-HT neurons were systematically suppressed during running in both positive and negative environments. Optogenetic manipulation of DRN GABA resulted in context-dependent behavioral changes while DRN 5-HT stimulation resulted in decreased speed in both contexts, consistent with the photometry data. These data support a major role for environmental valence in modulating dorsal raphe neural dynamics during active and inactive behaviors.

TOOLS FOR CIRCUIT MANIPULATION

71. **CW Berridge***, S Hupalo
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Cognitive and Neural Coding Actions of Corticotropin Releasing Factor (CRF) Neurotransmission in the Prefrontal Cortex
The prefrontal cortex (PFC) plays a critical role in higher cognition. We recently demonstrated that activation of corticotropin-releasing factor (CRF) receptors in the PFC of rats impairs, while CRF receptor blockade improves, working memory, similar to that seen with all approved ADHD drugs. These actions were topographically organized, being limited to the caudal dorsomedial PFC (dmPFC). To assess whether local CRF neurons are a prominent source of CRF for cognition-modulating receptors, we used a chemogenetic approach to selectively express excitatory (hM3Dq) or inhibitory (hM4Di) DREADDs (designer receptors) in rostral vs. caudal dmPFC CRF neurons. Activation of caudal, but not rostral, dmPFC CRF neurons impaired working memory, while suppression of these neurons improved working memory. These actions were prevented by local infusion of a CRF antagonist.
Additional studies examined the effects of PFC CRF neuronal activation on task-related activity of neurons within the dorsomedial frontostriatal pathway. Chemogenetic activation of CRF neurons in the dmPFC suppressed neurons

strongly tuned to key task events while having no significant impact on task-related activity of striatal neurons. Collectively, these observations demonstrate CRF neurons in the PFC exert robust modulatory actions on higher cognitive function that involve alterations in task-related neuronal signalling in the PFC.

72. **SM Conway***, MF Roitman
Graduate Program in Neuroscience, Dept. Psychology, University of Illinois at Chicago, USA

Chemogenetic inhibition of midbrain dopamine neurons suppresses amphetamine-induced dopamine transients
The mechanism by which amphetamine (AMPH) increases extracellular dopamine concentrations in the NAc is currently debated. Inconsistent with vesicular depletion and reverse transport characterized using in vitro preparations, AMPH increases the frequency of dopamine transients in vivo. Our study seeks to determine the extent to which AMPH-induced increases in NAc dopamine target phasic dopamine release signaled by neural activity at the dopamine cell bodies. We predicted that chemogenetic inhibition of VTA dopamine activation would attenuate AMPH-induced increases in NAc dopamine transients. To achieve inhibitory control of VTA dopamine neurons, we virally delivered the Cre-dependent inhibitory designer receptor exclusively activated by designer drug (DREADD) to the VTA of transgenic rats expressing Cre recombinase under control of the tyrosine hydroxylase promotor (TH:Cre+) and wildtype littermates (TH:Cre-). Using fast-scan cyclic voltammetry, we measured changes in the frequency of dopamine transients in the NAc in response to AMPH (2.5 mg/kg, IP). Similar to others, we observed AMPH-induced increases in dopamine transient frequency. This effect was significantly reduced exclusively in TH:Cre+ rats with CNO pretreatment (1 mg/kg, IP) relative to vehicle. Taken together, these results corroborate in vivo-based conclusions supporting VTA dopamine cell body activity as a critical target for AMPH action.

73. **AL Cremer[1]***, R Lippert[1], C Korn[2,3],T Jahans-Price[3], ME Walton[3], H Backes[1]
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Synaptic DA release induces low frequency variations in extracellular DA concentrations detectable by PET
Modelling results of [11C]raclopride (RAC) kinetics show that temporal variations of extracellular dopamine concentrations at time scales of minutes can cause detectable temporal fluctuations in the RAC PET signal. From theory, DA spillover after synaptic DA release can induce DA variations on these time scales. However, experimental evidence is still lacking.
We performed continuous fast-scan cyclic voltammetry measurements in a chemogenetic mouse model to test the theory. Dopaminergic activation led to an increase in the rate of spontaneous DA transients, as well as to an increase in the wavelet power of the signal at 0.5 Hz and at 0.01 Hz. The transient rate was strongly correlated with the wavelet power at 0.5 Hz reflecting the approximate duration of transients (~2 seconds). Both, transient rate and wavelet power at 0.5 Hz, significantly correlated with wavelet power at 0.01 Hz indicating that synaptic DA release indeed induced minute-scale variations of extracellular dopamine levels. By developing a new method for bolus-plus-infusion RAC-PET data analysis, we were able to assess temporal variations of the RAC signal as a measure for DA release in the same mouse model.

74. **JL Fudge***, Kelly EA
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Dopamine and CRF: broadening the view
Corticotropin-releasing factor (CRF), a neuroregulator of dopamine (DA) and mediator of stress responses, is produced in cell populations throughout the brain. Cross-talk between CRF and the midbrain DA system was identified several decades ago as a mechanism by which stress can influence motivated behaviors. For example, exogenous CRF and stress both trigger DA release in the rodent 'mesocorticolimbic' path, originating in the ventral tegmental area (VTA), to precipitate relapse to addiction. Yet, the midbrain DA neurons are increasingly understood to be highly heterogeneous, with neurochemical, physiologic and connectional diversity along their mediolateral and rostrocaudal axes, which map onto specific behaviors. Thus, DA neurons within and also outside of the 'classic VTA' contribute to a variety of motivated behaviours. We recently showed in primates that extended amygdala CRF-containing afferents terminate most densely outside the 'classic' VTA, among DA neurons associated with 'limbic associative' circuits, rather than pure 'mesolimbic' paths. Moreover, many potential afferent CRF sources outside the extended amygdala are positioned to innervate other DA subpopulations, and remain largely unexplored. We will discuss a broader concept of DA circuitry, including

DA subpopulation modulation by diverse CRF cell populations, can help pinpoint stressor impact on discrete DA subcircuits and behaviors.

75. **A Lak***, Harsha Gurnani, Miles Wells, Kenneth Harris, Matteo Carandini
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Projection-specific roles of dopamine neurons in decision making
Recent studies suggest that subpopulations of dopamine (DA) neurons with different projection targets play different roles in reward and action processing. To test the roles of these circuits in choice behaviour, we trained mice in a decision task and characterized the effects of optogenetic activation of DA neurons or their projection terminals on mice choices. In each trial, the headfixed mouse indicated the position of a stimulus appeared on the monitor by turning a steering wheel. We paired the water reward that followed one choice option with optogenetic DA stimulation. Following stimulation of VTA DA cell bodies or their terminals in ventral striatum, mice developed a tendency towards choices paired with such stimulations. This tendency affected the psychometric bias parameter, consistent with increased value of the stimulated choice option. In contrast, following stimulation of dorsal striatal DA terminals mice tended to take one action direction depending on the stimulated hemisphere. This tendency changed the psychometric lapse parameter indicating that dorsal striatal DA terminals are involved in response execution and not value processing. We propose a novel normative computational model that accounts for our observations. Together, these results illustrate distinct roles of subpopulations of DA neurons in decision making under uncertainty.

76. **R A Mohammed Jawad***, J Prados, C V Hutchinson
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Failure to Reconsolidate a Conditioned Place Preference in Planaria Treated with Atropine
It is known that retrieval of a memory requires a process of re-consolidation that involves a protein synthesis process; animals treated with a protein synthesis inhibitor during a test in which a previously learned association is evoked fail to retain this memory.
In the present experiments, brown planaria (Dugesia) were exposed to a 10% sucrose solution in one context in alternation with trials in which they were exposed to water in a different context. Test trials in which the animals could choose between the two contexts indicated the development of a conditioned place preference (CPP)—a preference for the context associated with sucrose. Repeated test trials in the absence of sucrose, however, led to the extinction of this CPP response. When the animals were exposed to sucrose in a novel context after extinction, the CPP response was successfully reinstated. However, a second group of animals treated with atropine (a muscarinic acetylcholine receptor antagonist) during the extinction trials failed to show reinstatement after been exposed to the sucrose rewarding agent.
This suggests that treatment with atropine prevented the re-consolidation process of the CPP memory when it was activated during the exposure to the context during the extinction phase.

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Two-photon uncaging of neuropeptides
Neuropeptides, which function in parallel with classical neurotransmitters, are implicated in cognition, sensorimotor processing and controlling blood flow. Although widely expressed in the brain, studying the effect of endogenously released neuropeptides in vivo has been hampered by inadequate techniques for controlling the release of neuropeptides. Here, we describe the development of a new optical tool for releasing neuropeptides with temporal and spatial precision. Specifically, we are developing two-photon (2p) uncaging of neuropeptides that are packaged inside nano vesicles, constructed from phospholipid liposomes coated with gold nanoparticles. Our preliminary data demonstrate in vitro uncaging of somatostatin (SST), which was monitored by a new cell-based neurotransmitter fluorescent engineered reporter (CNiFER) for SST. Furthermore, we have tested the 2p uncaging of a fluorescent dye (620 daltons) in vivo using nano-vesicles. Work is ongoing to investigate the in vivo uncaging and monitoring of SST and other neuropeptides. Controlling the release of neuropeptides in real-time in awake animals performing complex

behaviors would be transformative, enabling the elucidation of the function of neuropeptides in regulating neural circuits in the brain.

78. **RC Spencer***, CW Berridge
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Cognition Impairing vs. Cognition Improving Doses of Psychostimulants Target Different Aspects of Frontostriatal Neural Coding
The prefrontal cortex (PFC) and extended frontostriatal circuitry play a critical role in higher cognitive function. Dysregulation of PFC-dependent cognition is implicated in a variety of behavioural pathologies including addiction and ADHD. Psychostimulants are well known to exert dose-dependent cognitive actions. Specifically, at higher doses associated with psychostimulant abuse, these drugs robustly impair PFC-dependent cognition. In contrast, at low-doses used in the treatment of ADHD, these drugs improve PFC-dependent cognitive function. Currently, our understanding of the neural coding bases for these diverse cognitive actions of psychostimulants are unclear. To address this, we examined the effects of cognition-impairing and cognition-improving doses of methylphenidate (Ritalin) on task-related spiking activity of dorsomedial PFC and dorsomedial striatal neurons as well as functional connectivity between these regions as measured with local field potentials (LFP). Cognition-impairing doses of methylphenidate robustly suppressed the activity of both dorsomedial PFC and dorsomedial striatal neurons strongly tuned to key task events, while activating neurons not tuned to these events. Cognition-improving doses had minimal impact on task-related firing of either PFC or striatal neurons. However, in contrast to that seen with higher doses, cognition-improving doses of methylphenidate increased theta-related coherence between the PFC and striatum. These observations indicate that cognition-improving effects of psychostimulants target different aspects of neuronal coding than cognition-impairing doses.

79. **Y Van Den Herrewegen***, A Van Eeckhaut, D De Bundel, IJ Smolders
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Chemogenetic modulation of astrocytes in temporal lobe epilepsy
Presently available antiseizure drugs do not adequately control seizures in one-third of temporal lobe epilepsy (TLE) patients and innovative treatment options are therefore necessary. TLE is characterized by spontaneous epileptic seizures and severe comorbidities related to memory and mood. Lately, astrocytes are rising as potential treatment targets, since activated and proliferated astrocytes are a typical TLE hallmark. The combination of a clinically relevant mouse model for TLE, the intrahippocampal kainic acid mouse model, and a chemogenetic approach (Designer Receptor Exclusively Activated by a Designer Drug, DREADD) allows us to directly address the role of astrocyte specific signalling in TLE. Specific astrocyte modulation is obtained through stereotaxic infusion of an adeno-associated viral vector containing the inhibitory DREADD receptor, hM4Di, under the glial fibrillary acidic protein (GFAP) promotor. After systemic administration of the designer drug, the effect of astrocyte silencing on the recurrence of spontaneous seizures and comorbidities is assessed. With aid of continuous electroencephalography (EEG) monitoring and two behavioural tests, the sucrose intake test and the Barnes Maze, the amount of the seizures and the degree of anhedonia and cognitive impairment are determined. Specific astrocyte silencing could potentially result in seizure suppression and attenuation of the associated comorbidities.

MONITORING MOLECULES IN HUMANS AND TRANSLATIONAL APPROACHES

80. **H Backes[1]***, S Edwin Thanarajah[1,2], AL Cremer[1], R Lippert[1], C Korn[3,4], ME Walton[4]
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Novel raclopride PET method for spatiotemporal measurement of stimulus-evoked dopamine release in humans
Synaptic dopamine (DA) release induces temporal variations of extra-synaptic extracellular DA concentration on time scales up to minutes. These variations cause detectable temporal variations in [11C]raclopride (RAC) PET signal. From theory, the amplitude of RAC signal variations is directly proportional to the amount of released DA.

After validation of the theory in a chemogenetic mouse model, we performed bolus-plus-infusion RAC PET measurements in 10 healthy human volunteers under two conditions: once receiving milkshake and once tasteless solution during PET data acquisition. From PET data we calculated temporal variations of RAC to assess spatiotemporal distribution of dopamine release.
Distinct brain regions showed significant DA release at the time of food supply. 15-20 minutes after the food stimulus we identified further regions with delayed and presumably postingestive DA release. Moreover, DA release in other distinct brain regions at time of food intake was strongly correlated with the subjective "wanting" score, while the postingestive DA release in the putamen was negatively correlated with "wanting". We hypothesize that DA release in the "wanting system" at the time of food intake inhibits postingestive dopaminergic signalling.

81. **Bronzuoli MR[1]***, Facchinetti R[1], Cassano T[2], Steardo L[1] Scuderi C[1]
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Astrocytes and Alzheimer's disease: the pharmacological manipulation as promising tool against pathology progression. Evidence from a triple transgenic model of the disease
Alzheimer's disease (AD) is a serious health and economic challenge. So far, available treatments provide only symptomatic relief, making necessary a multitargeted approach against the several pathological processes underlying such disease. In particular, Aβ- and tau-pathology, astrocyte dysfunction, neuroinflammation and glutamate unbalance are the main targets to counteract. To this aim, palmitoylethanolamide (PEA) can be considered a multitargeted treatment strategy. Here, we tested the effects of a 3-months treatment with ultramicronized PEA (um-PEA) in young (6-month-old) and adult (12-month-old) 3×Tg-AD mice, compared to their age-matched Non-Tg mice. In particular, potential neuropathological mechanisms were assessed by western blot, RT-PCR, and immunofluorescence in the hippocampal tissues. Our finding revealed that um-PEA normalizes astrocytic functionality, rebalances the astrocyte glutamate regulating system, restrains neuroinflammation and promotes neuronal survival. Such um-PEA efficacy is potent especially in younger mice, suggesting its potential as a precocious treatment. Since PEA is already licenced for use in humans, displaying a high tolerability and safety profile, it would be an ideal candidate for a long-term use lasting several years, as potential AD treatment requires.

82. **I. Betina Ip[1,2]***, Andrew J. Parker[1], Uzay E. Emir[2,3], Holly Bridge[2]
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Imaging dynamic neurochemical signals to visual contrast in the human brain using 7 Tesla MR Spectroscopy
Functional Magnetic Resonance Imaging is one of the most widely used non-invasive measures of human brain function. It uses the Blood-Oxygenation-Level-Dependent (BOLD)-signal as a metric of neural activity. A key challenge to the field is that the BOLD-signal is ambiguous regarding the relative contributions of neuronal excitation and inhibition. We aimed to address this challenge by using complementary MR imaging methods, fMRI and 1H-Magnetic resonance spectroscopy (MRS), to identify simultaneous changes in hemodynamics and neurochemistry as a function of visual contrast levels. Participants viewed 64-sec stimulus blocks of flashing checkerboards at four stimulus contrasts (3, 12.5, 50, 100%), alternating with a blank mid-gray screen. Combined fMRI-MRS data were acquired from a 2x2x2 cm3 voxel in the primary visual cortex. Increase in stimulus contrast evoked a linear increase in BOLD-signal. In addition, the response of major excitatory neurotransmitter glutamate increased with contrast whereas inhibitory neurotransmitter γ-aminobutyric acid responses decreased. Quantification of this change using an excitation:inhibition index demonstrated a switch from inhibitory-dominant to excitatory-dominant neurochemical response. In summary, we have identified a dynamic interplay between excitation and inhibition dependent on the response strength in the primary visual cortex. Our results are a step towards disambiguating contributions of cortical excitation and inhibition to stimulus evoked hemodynamic response.

83. **JH Kim[1]***, SW Park[1], JH Yun[2], JW Kim[1], CH Cho[2], JH (Jeong Hun) Kim[1,2,3]
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Angiopoietin 2 as an early marker of blood-retinal barrier breakdown in diabetic retinopathy induces astrocyte apoptosis via αvβ5-integrin signaling pathway

The vascular leakage in diabetic retinopathy is followed by blood-retinal barrier breakdown and it leads to macular edema and vision loss. Although astrocyte play an important role in regulating blood-brain barrier integrity in the brain, the precise role of astrocyte in blood-retinal barrier was yet to be elucidated. This study aimed to investigate the role of angiopoietin 2 (Ang2) in astrocyte loss and vascular leakage in the early streptozotocin-induced diabetic retinopathy. We demonstrated that vascular leakage occurred with astrocyte loss in early diabetic mice retina as Ang2 increased. The astrocyte loss and vascular leakage were inhibited by intravitreal injection of Ang2-neutralizing antibody. In vitro, Ang2 aggravated high glucose-induced astrocyte apoptosis via GSK-3β activation. Ang2 directly bound to αvβ5 integrin, which was abundant in astrocyte, and the blockade of αvβ5 integrin, in vitro, effectively attenuated Ang2-induced astrocyte apoptosis. In vivo, intravitreal injection of anti-αvβ5-integrin antibody inhibited astrocyte loss in early diabetic retinopathy. Taken together, Ang2 induced astrocyte apoptosis under high glucose via αvβ5-integrin/GSK-3β/β-catenin pathway. Therefore, we suggest that Ang2/integrin signaling could be a potential therapeutic target to prevent the vascular leakage by astrocyte loss in early diabetic retinopathy.

84. **C Lang***, K Campbell, B Ryan, C Webber, R Wade-Martins
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Single cell sequencing reveals HDAC4 as a regulator of cellular phenotypes in Parkinson's iPSC-derived dopamine neurons

Patient obtained induced pluripotent stem cell (iPSC)-derived dopamine neurons provide an opportunity to model Parkinson's disease (PD) in previously inaccessible neurons, which recapitulate the genetic background of patients. However cell cultures are notoriously confounded by cellular heterogeneity. By isolating dopaminergic neurons from these cultures and applying high-resolution single cell and bulk RNA-sequencing transcriptomic analyses to PD iPSC-derived dopamine neurons, we exploited intra-culture cellular heterogeneity to identify a progressive axis of gene expression variation within the dopaminergic neuron cell population. Analysis of genes differentially expressed (DE) early across this axis identified the transcriptional repressor, histone deacetylase 4 (HDAC4), as an upstream regulator of disease progression. HDAC4 was observed as mislocalised to the nucleus in PD iPSC-derived dopamine neurons and repressed genes early in the disease axis, leading to later deficits in ER stress. Treatment of neurons with compounds, re-localised HDAC4, activated previously repressed early DE genes and corrected a number of PD-related cellular phenotypes, including ER stress, autophagy perturbation and alpha synuclein release. Our study demonstrates how single cell RNA-Sequencing can exploit iPSC cellular heterogeneity for patient stratification and to reveal disease mechanisms, which can be used to identify potential targets and repurposed therapeutics of interest in the treatment of PD.

85. **MAG Martens[1]***, A Antley[1], M Slater[2], D Freeman[1], EM Tunbridge[1,3], PJ Harrison[1,3]
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The psychological and physiological effects of a novel virtual reality stressor

We investigated the physiological and psychological effects of a novel virtual reality (VR) environment laboratory-based stressor.

Non-smoking, healthy men (N=12 per group, mean age=24.9 years, SD=4.2) attended the VR lab and, following a one-hour baseline period, were randomly assigned to either the control or stressor version of a VR elevator environment. Physiological measures of arousal (salivary cortisol and amylase, blood pressure and pulse (monitored for 30 seconds)), and subjective stress ratings, were recorded at 20 minute intervals. Skin conductance level was measured continuously. Data was expressed and analysed as percentage change from baseline, and compared between the groups immediately after stress induction, using t-tests or Mann-Whitney test as appropriate.

Pulse, skin conductance, salivary cortisol and amylase, and subjective stress ratings, increased to a greater extent in the stress condition than the control condition. There were no group differences in blood pressure.

Exposure to the VR stressor led to increases in physiological and subjective stress. Our findings demonstrate that experiences in a VR environment can induce robust physiological responses and suggest that VR may prove to be useful for studying the acute effects of drugs on the stress response.

86. **IC Samper***, SAN Gowers, ML Rogers, MG Boutelle
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Robust high-resolution 3D printed microfluidic device for online monitoring of dynamic events in the injured human brain

The recent consensus statement from the 2014 International Microdialysis Forum [1] identified the need for continuous real-time chemical monitoring of traumatic brain injury patients. Spreading depolarisations (SDs) are important secondary insults that place an extreme dynamic challenge on the energy supply to the tissue. Propagating through the cortex and around the injury, SDs are associated with poor patient outcome [2]. On-line neurochemical monitoring of energy availability in "at risk" tissue enables detection of the metabolic consequences of SDs giving a measure of the expansion of the injury.

We have developed a system in which the dialysate stream continuously flows into our newly engineered analyser. Our system consists of a high-resolution 3D printed microfluidic flow cell. It incorporates removable, integrated biosensor-based amperometric detectors for glucose, lactate and glutamate, each based inside a 400 μm needle.

Optimisation of the 3D printed channel size and resulting improvement in sensor response time will be presented here together with our recent advances in glutamate sensing. Neurochemical changes in the injured brain were simulated using an automated microfluidic board and recorded with this novel device. These results will be presented together with preliminary patient data.

1. Hutchinson et al. Intensive care Med. DOI: 10.1007/s00134-015-3839-5 (2015)
2. Hartings et al. The Lancet Neurology, 10, 1058–64 (2011)

87. **N Zarghami***, M Sarmiento Soto, NR. Sibson
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Activated leukocyte cell adhesion molecule (ALCAM) as a potential imaging biomarker for detection of brain micrometastases

The incidence of brain metastasis is increasing. Diagnosis and treatment of these tumours at early stages is challenging since the intact blood-brain barrier limits access for both imaging contrast agents and systemic therapies. However, new molecularly-targeted magnetic resonance imaging (MRI) contrast agents could enable early detection of metastases and, thus, open a window of opportunity for earlier treatment. The aims of this study are: (1) to evaluate activated leukocyte cell adhesion molecule (ALCAM) as another potential target for brain micrometastasis detection; and (2) to develop a new MRI contrast agent based on iron oxide (MPIO) and anti-ALCAM antibodies.

In vitro experiments demonstrated marked ALCAM upregulation on mouse endothelial cells following treatment with tumour conditioned media from metastatic breast cancer cells. Subsequently, immunohistochemistry of both mouse and human brain metastasis tissue showed ALCAM upregulation within the tumour microenvironment and on associated vessels.

Antibodies against ALCAM were successfully coupled with MPIO. Specific binding of the ALCAM-MPIO to ALCAM was tested in vitro endothelial cells, previously exposed to IL-1β, and compared to MPIO conjugated to an equivalent, but non-specific IgG antibody. Pilot in vivo MRI studies have shown promising results.

If successful, clinical translation of this approach could substantially improve therapeutic options for patients at risk of brain metastasis.

88. **Liu Shi[1]***, Sarah Westwood[1], Alison Baird[1], Sneha Anand[1], Abdul Hye[2], Isabelle Bos[3], Stephanie Vos[3], Rik Vandenberghe[4], Philip Scheltens[5], Sebastiaan Engelborghs[6,7], Giovanni Frisoni[8], José Luis Molinuevo[9,10], Anders Wallin[11], Alberto Lleó[12], Julius Popp[13], Pablo Martinez-Lage[14], Stuart Snowden[2], Cristina Legido-Quigley[2], Lars Bertram[15], Frederik Barkhof[16], Henrik Zetterberg[17,18,19,20], Johannes Streffer[21], Pieter Jelle Visser[3,5], Simon Lovestone[1]

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Alzheimer's disease plasma proteomic biomarker validation using SOMAscan technology: a systematic review and replication study

Introduction: A systematic review of the literature revealed that numerous plasma proteins were reported associated with Alzheimer's disease (AD) pathology including amyloid PET, cerebrospinal fluid (CSF) levels of amyloid-β (Aβ), tau and brain atrophy, as well as progression from prodromal disease to AD. The further validation of these candidate biomarkers in a large cohort is critical. Therefore, we aimed at validating previously reported proteomic biomarker signatures in a large cohort by using a multiple protein technology- SOMAscan.

Methods: We firstly searched the literature and summarized the reported plasma proteomic biomarkers which are associated with AD pathology and disease progression. We then used SOMAscan proteomics platform to screen more than 593 plasma samples from IMI-European Medical Information Framework (EMIF). Finally, we chose the same panel of proteins, analysed them in the same statistical approach, and compared the results with literature.

Results: In total, there were 36 eligible studies reporting different panels of proteins reflecting AD pathology. Results showed that we could not generate exactly the same results as reported in the literature. This might be due to the difference of proteomic methods used, samples origin, sample size etc. However, several proteins were associated with AD pathology and replicated previous studies.

MICRODIALYSIS, EXTRACELLULAR ANALYSIS, RELATED METHODS

89. **Thomas Birngruber[1]***, Thomas Altendorfer-Kroath[1], Srini Jayaraman[3], Frank Sinner[1,2]
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Cerebral open flow microperfusion – a sampling tool for longerm monitoring of transport across the BBB

A recently developed sampling technique called cerebral open flow microperfusion (cOFM) enables sampling of brain interstitial fluid and CSF and provides long term access directly to the brain tissue bypassing the BBB. cOFM probes feature macroscopic openings for the exchange of substances with the cerebral ISF. This open exchange structure avoids membrane-related problems such as biofouling, protein clotting, high molecular weight cut-off and the exclusion of large and lipophilic substances. Similar to other probe-based sampling techniques, cOFM probe implantation causes capillary rupture and thus disruption of the BBB. As an intact BBB is necessary to assess substance transport across the BBB, cOFM studies implement a 14 day healing period to ensure re-establishment of the BBB. Most probe-based sampling technologies that use implanted probes for measurement and sampling in the brain are limited in application time due to the formation of a glial scar that leads to encapsulation of the probe. The design of the cOFM probe and especially the used materials are optimized to evoke minimal tissue reaction. No continuous glial scar was found up to 30 days after cOFM probe implantation when glial scarring was measured by qualitative and quantitative histological tissue analysis of microglia and astrocytes.

90. **R Colbourn[1]***, J Goodman[2,3], S Hrabetova[4]
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Dynamic Volume Changes of the Brain's Extracellular Space Underlying Seizures

The brain's extracellular space (ECS) is known to play a critical role in determining the excitability of neurons, and through this function, promote or inhibit seizure activity. It has been established that the ECS undergoes a long-lasting

shrinkage of about 30% during a seizure. However, this project investigates a previously unreported phenomenon: the ECS undergoes a fast shrinkage, then slow expansion back to baseline volume during each synchronous neuronal discharge that occurs during epileptiform activity. The goal of this project is to establish these dynamic volume changes (DVCs) as a mechanism that promotes seizure activity and determine if manipulation of this phenomenon halts seizures. The first experiments that characterized DVCs during drug-induced epileptiform activity in mouse neocortical slices revealed that DVCs lead to a transient 22% reduction in ECS volume. This observation has also been repeated in vivo through rat neocortical measurements of ECS volume during drug-induced epileptiform activity. Because water transport between the intra- and extra-cellular compartment is likely responsible for DVCs, pharmacological blockade of osmotic and ionic transport proteins was performed, revealing several protein dependencies of seizures and their DVCs. These channels and others yet to be investigated may represent new therapeutic targets to treat seizures.

91. **M Eysberg***
Antec Scientific, Netherlands

Method development in neurotransmitter analysis to improve selectivity, sensitivity and robustness
In vivo microdialysis has become an invaluable tool that provides real-time information of neurotransmitter levels in living brain. Microdialysis samples are collected and stored, or analyzed immediately in an on-line configuration using UHPLC-ECD. It is an analytical challenge to provide reproducible and accurate data as neurotransmitter levels are often below picomolar concentration range.
To get as much information from samples <5ul, improvement of selectivity, sensitivity and robustness is necessary. To improve and obtain this, chromatography aspects e.g. high efficiency columns, flow cell configurations, etc are studied and evaluated.
The ALEXYS Neurotransmitter analyzer comprises a number of integrated system solutions, which have been developed for trace analysis of neurotransmitters. Parallel and serial detection schemes using multiple flow cells have been used instead of running sequential trials for different neurotransmitters. Dual or triple loop injection valves are applied with minimum sample consumption on parallel UHPLC systems under completely different conditions. Getting more information out of fewer samples is not only saving time and money but - in the end - also rodents.

92. **M Eysberg***, LM van Heerwaarden, H-J Brouwer, N Reinhoud
Antec Scientific, Netherlands

ALEXYS Neurotransmitter Analyzer
Neurotransmitters from the brain can be sampled by in-vivo techniques such as microdialysis or by in-vitro techniques such as brain tissue homogenates. In both cases, sample volumes are small when high time resolution or spatial resolution is required. As concentrations of neurotransmitters are generally low, this puts a high demand on the detection sensitivity and on the handling of small sample volumes with the analytical instrumentation. The ALEXYS Neurotransmitter Analyzer is a versatile and flexible UHPLC platform with electrochemical detector that is capable to analyze such samples with the best possible sensitivity. In this poster, we present an overview of the optimized methods for analyzing monoamines, acidic monoamines, acetylcholine and choline, GABA, glutamate and other amino acids with the ALEXYS Neurotransmitter Analyzer.

93. **J Hrabe[1,2]***, R Colbourn[3], S Hrabetova[2]
[1]Medical Physics Laboratory, Nathan Kline Institute, USA; [2]Department of Cell Biology, State University of New York Downstate Medical Center, USA; [3]School of Graduate Studies, State University of New York Downstate Medical Center, USA

Monte Carlo models of dynamically changing extracellular space
Brain extracellular space (ECS) serves as a channel for signaling molecules and metabolites. ECS volume impacts concentrations of substances and thus their action. The volume can change dynamically on time scales ranging from many hours (e.g., the sleep-wake cycle) to a few seconds (e.g., the epilepsy). Using MCell (http://www.mcell.org), we numerically model the impact of ECS volume dynamics on spatiotemporal distribution of chemical signals at different spatial scales.
On a very local scale, modeling illustrates conditions under which dynamic ECS volume changes influence molecular concentrations adjacent to moving cell membranes. Like a speeding car windshield gathering raindrops, a membrane moving through a background concentration of molecules creates a pseudo source at the membrane. Such a source transiently increases the probability of interactions with appropriate membrane receptors.

On a larger spatial scale, modeling explains experimentally observed pulsatile ECS concentrations in hippocampus, coincident with epileptic activity. We show that the dynamic ECS volume changes are likely restricted to the stratum pyramidale layer, which then acts as a transient macroscopic diffusion source supplying molecules to the surrounding structures.

94. **A Jaquins-Gerstl***, KT Ngo, AC Michael, SG Weber
Department of Chemistry, University of Pittsburgh, USA

Toward Monitoring Dopamine Transients using Fast Microdialysis Liquid Chromatography with Electrochemical Detection

A mainstay in neurochemistry research is the technique microdialysis. It is recognized for its breadth of application and ease of use. Despite the many advantages of microdialysis it has a major disadvantage; poor temporal resolution. This is critical, poor time resolution can lead to misleading results in behavioral experiments. Our primarily goal is to improve dopamine measurements by carrying out experiments on a faster timescale than that currently practiced. We have developed fast microdialysis liquid chromatography with electrochemical detection (fmLC-EC) to determine when the dynamic shift in dopamine signaling occurs in animals undergoing a learning paradigm. To date, this has only been possible with fast scan cyclic voltammetry.

In this work we performed in vivo online fmLC-EC to monitor dopamine transients in response to food-reward-cue experiments with a 45 second temporal resolution in the striatum of freely moving rats. Optimizations of chromatographic conditions were carried out with emphasis on temporal resolution. Retrodialysis of dexamethasone was used to minimize tissue disruption due to probe implantation Using fmLC-EC we hypothesize that in time, after repeated cues/reward parings DA signals shift to predictive cue onset and not that of reward. Our ability to improve approaches for determining extracellular dopamine is significant.

95. **F Le Prieult[1]***, K Buck[2], M Mezler[1], L Laplanche[1]
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Brain bioavailability of large molecules in rodents

Measuring and understanding concentrations of neuroactive drugs in the interstitial fluid (ISF) of the brain is a topic of great significance in the field of pharmacokinetics (PK) and pharmacodynamics (PD). Due to the fast-growing development of therapeutic monoclonal antibodies for neurodegenerative disease, methods for sampling extracellular high molecular weight proteins have become of particular interest. Tackling this subject, large pore in vivo microdialysis along with open flow microperfusion offers great promise in understanding the PK/PD relationship of therapeutic proteins. We analyzed the brain delivery of the antibody Trastuzumab as a first model molecule, and showed that the ISF concentration in mouse brain was approximately 1% of plasma. Steady-state levels in ISF were reached rapidly (after approximatively 10h), indicating fast penetration, and reached a steady plateau across the measurement period of 48 hours. This sampling method opens possibilities for direct measurement of pharmacologically relevant concentrations in ISF, and will furthermore enable a pharmacokinetic comparison of novel antibodies targeting the central nervous system, including molecules with specific properties, for example enhancing their penetration across the blood-brain barrier.

96. **E Barini[1]***, M Meinhardt[1], T Altendorfer-Kroath[2], J Hoppe[1], G Plotzky[1], I Mairhofer[3], F Le Prieult[3], M Mezler[3], HJ Mayer[4], L Gasparini[1], T Birngruber[2], K Buck[1]
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Sampling extracellular Tau in human Tau transgenic mice: optimization of push/pull in vivo microdialysis

A key neuropathological hallmark of Alzheimer disease is the presence of intracellular filamentous inclusions of the microtubule-associated protein Tau. Emerging evidence suggests that AD progression is mediated by cell-to-cell transfer of Tau, which involves its release into the extracellular space. Our goal was to further advance the push/pull

microdialysis technique to sample extracellular Tau from the brain of human Tau overexpressing mice and compare it to cerebral open flow microperfusion (cOFM).
As first step towards optimizing the push/pull microdialysis setup, we implemented an online flow sensor for precise monitoring of the flow rate at high resolution to ensure stable push/pull conditions throughout the experiment. Then, we investigated Tau in vitro recovery using different tubing materials, perfusion fluids with various additives (BSA, pluronic, Tween) and probe types. This optimized setup has been used to analyze basal and K^+-induced Tau release in rTg4510 mice bilaterally implanted with Eicom microdialysis probes (1MDa cut-off). We found that basal levels of extracellular human Tau decrease during Tau pathology progression, but remain responsive to modulation by neuronal activity. Furthermore, a study is currently ongoing to investigate basal and K^+-evoked Tau release in rTg4510 animals implanted with cOFM probes, which adds several advantages compared to conventional microdialysis.
Disclosures:
MM, JH, GP, IM, MM, HJM, LG and KB are employees of AbbVie. TAK and TB are employees of the Joanneum Research GmbH. The design and study conduct were provided by both Joanneum Research GmbH and AbbVie. The financial support for this research was provided by AbbVie. Both AbbVie and the Joanneum Research GmbH participated in the interpretation of data, review, and approval of the publication.

97. **A Morales-Villagrán***, K Pardo-Peña
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Glutamate measurement online and at high temporal resolution, using a new microdialysis procedure and an optic device
The classic microdialysis method lacks of high temporal resolution and is mainly coupled to HPLC. In this work, a new alternative is proposed to improve this drawback and thus obtain a better understanding of the dynamics of Glutamate or other molecules of biological interest. The new set up was designed and built to measure hydrogen peroxide as a product of reaction of Glutamate oxidase, which is quantified by the Amplex Red method. The device consists in a fluorescence cell in which the fluid coming from the microdialysis is mixed online with an enzymatic reactor containing Glutamate oxidase and Amplex Red, then this mixture goes into the cell and the fluorescence is measured at sub-seconds time resolution, which is proportional to the Glutamate concentration. To test the reliability of this method, calibration curves for Glutamate were run resulting in a linear response ($R \geq 98$) and then a microdialysis probe was placed into the hippocampus to measure the Glutamate during seizure induced by pentilenetetrazole, the results showed a relation between the extracellular increase in this neurotransmitter and the seizure activity. This method can be used to measure other compounds that generate fluorescence with Amplex Red or any other fluorescence probe.

98. **KT Ngo***, A Jaquins-Gerstl, SG Weber
Department of Chemistry, University of Pittsburgh, USA

Understanding the Limitations to Better Time Resolution in Microdialysis
Using microdialysis/capillary liquid chromatography (cLC) we have developed online one-minute measurements of dopamine (DA) and, separately, serotonin. As separation speed improves, it is appropriate to consider the effect of the probe and fluidic connections on resolution (expressed as a standard deviation, s, in s). In vitro, a step concentration change in DA outside a probe permits assessment of s. Online cLC with 45 or 60 s chromatograms quantitates DA. A time profile of the resulting step is created by repeating the step change with different delay times between step and cLC injection. With current home-made probes, s for the probe + connection tubing + cLC injection loop is about 15 s. Adding a retrodialysis injection valve and inlet capillary upstream of the probe gives $s \approx 17$ s. The time delay between the concentration change and its detection was $t1 = 8.4$ min. We are currently evaluating the prospects for a rapid no-net-flux experiment in awake rats which would be a boon to researchers. Our current measurements (striatal dopamine) determine probe recovery and external concentration from 4.5 min step concentration changes. We are also attempting to deduce neurochemical responses to step concentration changes by deconvoluting in vivo and in vitro responses.

99. **JC Salazar-Sánchez***, A Morales-Villagrán
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GABA quantification by an electrochemiluminescence method using enzymatic reactors
γ-Aminobutyric Acid (GABA) is well known as a neurotransmitter which regulates inhibitory neurotransmission in mammalian central nervous systems (CNS), but also participates in several processes outside the brain. However, measuring GABA, entails several challenges because it is neither fluorescent nor electroactive and it is difficult to detect by enzymatic reactions because no oxidases or dehydrogenases have been found. Several methods have been developed to quantify GABA based on the instrumentation available, the sensitivity required, and the volume of samples analyzed. Most of these methods use HPLC. Here we describe GABA quantification in small volume samples through enzymatically induced electrochemiluminescence, obtained using the well-known GABAse complex, which produces glutamate (Glu) that can be used to generate a luminescent reaction by means of Glutamate oxidase (GluOx) and Luminol in an electrochemiluminescence cell. The luminescence obtained was proportional to the GABA concentrations in the micromolar range (1-1000), with linear r2 values > 0.95. To validate this procedure, GABA standards were treated with the enzymatic reactors to generate Glu, which was measured simultaneously with a HPLC technique. This approach is a good alternative for monitoring GABA with good sensitivity compared with traditional methods still in use.

100. **SA Shippy***, P Fisher
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Miniaturized push-pull perfusion sampling of hippocampal slices
Techniques to sample the extracellular space provide a distinct compatibility for measurement of varied chemical content. The miniaturization of sampling probes will provide advantages both for both increased sampling spatial resolution and reduced damage to tissue. A miniaturized push-pull probe is described in this work that has a pulled tip size on the order of single microns. Concentric, fused-silica capillary-constructed push-pull probes are pulled in a home-built, flame-based, gravity puller to fabricate single micron tips. Tip size is measured with optical and electron microscopies. Infusion and withdrawal flows are calibrated for 10-20 nL/min perfusion rates similar to larger, low-flow push-pull perfusion probes. Probes are tested in mouse hippocampal slices to characterize glutamate content via a capillary electrophoresis assay. While miniaturized probe backpressures are somewhat increased compared to low-flow push-pull perfusion, vacuum withdrawal flow rates are easily realized. In vitro testing shows greater than 90% recoveries of standards amino acids. Ex vivo sampling from hippocampal tissues demonstrates low micromolar concentrations and a loss of glutamate from slices over time. Comparison of amino acid levels over 2 hours of sampling does not show evidence of tissue damage with probe placement. Experiments for combined sampling and electrophysiology are discussed.

101. **RE Wilson***, A Jaquins-Gerstl, SG Weber
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On-Column Dimethyl Labeling of Neuropeptides with Online Microdialysis and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS2)
Neuropeptides are important for cell signalling but are difficult to study due to low concentrations in the extracellular space. MS2 offers the high sensitivity required for these studies but non-volatile salts in the perfusate interfere with ionization. Thus, robust quantitation requires internal standards, ideally isotopic analogues of the analytes of interest. This often limits LC-MS analyses to offline methods, which can lead to sample degradation and contamination. We have adapted a proteomics-based multiplexing technique developed by Raijmakers, et al. to the conditions and timescale required for the online detection of peptides in rat brain microdialysates. Neuropeptides are trapped on a C18 column and reduced and dimethylated at the N-termini using cyanoborohydride and formaldehyde ("light"). Peptide standards are then injected onto the column and labelled with formaldehyde-d2("heavy"), resulting in Δm/z of 4. After labelling, both heavy and light peptides are simultaneously eluted off the column and analysed by MS2. Comparison of light and heavy peptide peak areas allows correction for matrix effects. Using this technique to measure relative changes of leucine-enkephalin in the rat hippocampus, we have demonstrated a completely automated and versatile approach for online quantitation of endogenous neuropeptides in brain microdialysates.

102. L Denoroy, **S Parrot***
Inserm UMR1028, Lyon Neuroscience Research Centre, NeuroDialyTics Unit & BioRaN Team, University Lyon 1, France

Advances and pitfalls in the capillary electrophoresis analysis of aggregates of beta amyloid peptides
Alzheimer's disease is characterized by the accumulation of brain amyloid plaques due to the progressive deposition of aggregates of amyloid β (Aβ) peptides. The present work describes a novel and easy-to-run method based on capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) for the specific analysis of fibrillar forms of Aβ aggregates obtained after in vitro incubation of Aβ 1-40 monomer. For that purpose, an affinity CE-LIF approach, in which thioflavine T is added as a ligand in the running buffer, has been used. Under such conditions, various fibrillar aggregates migrate and are detected as spikes. The procedure has been optimized to get spikes corresponding only to Aβ aggregates, through the careful elimination of interfering factors and the electrophoretic validation of the link between spikes and particulate material. Our method exhibits quantification capabilities, leading to the separation of Aβ fibrillar aggregates of different sizes and allows to show that high concentrated solutions of Aβ peptide form aggregates of larger size than lower concentrated solution do. Advances brought by this method as well as future development needed to overcome its limitations are discussed.

MICRODIALYSIS: FUNCTIONAL STUDIES

103. **S Parrot***, S Aboudiaf, L Seugnet.
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LAT- like amino acid transporters regulate dopaminergic transmission and sleep in Drosophila
We use Drosophila to identify and study genes affecting monoamines transmission and sleep-wake regulation. Sleep and wakefulness are strongly modulated by dopamine, one of the major wakening neurotransmitters across the animal kingdom. Here we investigated the role of neuronal LAT1-like amino-acid transporters on dopaminergic function. LAT1-like transporters are involved in the uptake of essential amino acids required for dopamine synthesis, translation and energy metabolism. LAT1 can also transport L-DOPA, a dopamine precursor used to treat Parkinson's disease. Two Drosophila LAT1-like transporters, JhI21 and mnd, were downregulated in dopaminergic neurons using transgenic constructs. The mutant flies were less awake and less responsive to L-DOPA, indicating of dopaminergic transmission defects. To determine whether these effects were linked to reduced dopamine levels we quantified dopamine in single brains using capillary HPLC and electrochemical detection, and correlated it with individual sleep data. Under baseline conditions mutant and control flies showed similar dopamine levels. As expected dopamine levels were increased after L-DOPA intake, and again mutant flies appeared as responsive as controls. L-DOPA feeding also resulted in the detection of noradrenalin in the brain, not normally synthesized in Drosophila. These results indicate that LAT1-like transporters impact dopaminergic transmission, independently of a change in dopamine levels.

104. **S Parrot1***, D Dinca2,3, S Braz2,3, B Potier4, A Huguet-Lachon2,3, P Dutar4, G Gourdon2,3, M Gomes-Pereira2,3
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Alteration of brain glutamate transport in a mouse model of myotonic dystrophy type 1 (DM1)
Myotonic dystrophy type 1 (DM1) is a genetic disorder with symptoms such as muscle weakness and myotonia, but also intellectual and behavioural deficits. The disease is progressive and leads to significant disability. The mutation causing DM1 has been identified as unstable expanded CTG repeats: from 50 to >4000 CTG trinucleotides, instead of normal <37 CTG repeats. The size of the CTG repeats increases from generation to generation, as well as with age in many tissues, including brain. To elucidate the mechanisms behind CNS dysfunction and find therapies targeting DM1, the model of homozygous DMSXL mice (>1000 CTG), exhibiting significant cognitive, behavioural and electrophysiological deficits similar to the most severe form of the human pathology, was used. Proteomics studies carried out in DMSXL brains, revealed expression abnormalities of glutamate transporters more pronounced in astrocytes that in neurons, suggesting a strong impact on glutamate homeostasis. The expression of glutamate transporters (GLT-1), the ability of glutamate uptake and its consequence on glutamate levels and neurotransmission are now evaluated in hippocampus and cortex, using western blot, in vitro isotopic uptake measurements, but also in vivo microdialysis and whole-cell patch-clamp recordings. The first results show a decrease in GLT-1 protein levels accompanied by glutamate neurotoxicity in culture and glutamatergic alterations in vitro.

105. **EV Efimova[1]***, KA Antonova[1], M Ptuha[2], AB Volnova[2], RR Gainetdinov[1]
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Action of TAAR5 agonist alpha-NETA on brain monoamine systems

Trace amines are structurally close to classical monoamine neurotransmitters and they play an important role in regulation of movement, feeding and many other functions. However many of other functions in mammals are still remain unknown. Unraveling the role of trace amine in physiology could give answers to pathologies and pharmacology of monoamine neurotransmission. Mostly studied is TAAR1 receptor, whereas for other receptors still little information is known. In our laboratory we identified new agonist for TAAR5 receptor, alpha-NETA. Study of action of TAAR5 agonist can elucidate the functions and modulatory influence of trace amines on classical neuromediators. We analyzed the characteristics of the power spectrum of ECoG signal recorded in freely moving mice. a-NETA at a dose of 5 mg kg significantly increased the power of the ECoG signal in the delta frequency range (2.5-4.5 Hz). To analyze action of alpha-NETA on monoamine system we measured level of dopamine, serotonin and their metabolites using HPLC and also performed microdialisys in striatum for 2 hours after injection. Our study revealed that TAAR5 agonist alpha-NETA is modulating activity on monoamine systems, increasing total dopamine level and decreasing dopamine/DOPAC ratio.

106. **B Ferger[1]***, G Flik[2], R Arban[1], S Hobson[1], C Dorner-Ciossek[1]
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In vivo microdialysis study of a procognitive phosphodiesterase-2 inhibitor in rat prefrontal cortex, striatum and hippocampus

Pharmacological inhibition of phosphodiesterase-2 (PDE2) is thought to be procognitive and to functionally improve glutamatergic neurotransmission.

This is the first in vivo microdialysis study to investigate the selective PDE2 inhibitor PF-05180999 (3, 10, 30 mg/kg, po) on multiple neurotransmitters and cyclic nucleotides. Microdialysis probes were implanted in the prefrontal cortex (PFC), ventral striatum or hippocampus of Wistar rats. Acetylcholine, dopamine, GABA, noradrenaline, 5-HT and glutamate were measured in the PFC and ventral striatum. Glutamate was additionally quantified in the hippocampus. As target engagement marker cGMP and cAMP were determined. All microdialysates were analysed by HPLC with tandem mass spectrometry.

As expected the absolute basal neurotransmitter levels were different according to the brain structure measured. The lowest absolute basal concentration was obtained for cGMP and cAMP (0.05 and 0.12 nM, respectively). After 60-120 min PDE 2 inhibitor treatment revealed a maximum effect on cGMP (400% increase) in the PFC. Neither glutamate nor any other neurotransmitter was significantly altered after PDE2 inhibitor treatment.

In conclusion, using a highly sensitive analytical method in combination with in vivo microdialysis cGMP was the most sensitive biomarker to indicate target engagement of PDE2 inhibition in the PFC in a time dependent and dose-related manner.

107. **J Kehr[1,2]***, T Yoshitake[2] , FH Wang[1], S Schmidt[1], P Banerjee[3], J Donello[3]
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Rapastinel antidepressant-like activity is independent of increased efflux of dopamine, 5-HT and glutamate as observed for S(+)-ketamine in the rat mPFC

Rapastinel (Glyx-13) is a novel NMDA receptor modulator with glycine-like partial agonist properties. Rapastinel is in late-stage clinical development as an adjunct therapy for major depressive disorder. Both rapastinel and ketamine produce antidepressant-like effects in rodent models of depression but rapastinel does not exhibit ketamine-like CNS adverse effects. In the present study, we evaluated acute effects of rapastinel and S(+)-ketamine administered at doses effective in the rat forced swim test (FST), on extracellular levels of dopamine (DA), 5-HT and glutamate in the rat mPFC using intracerebral microdialysis. Microdialysis (Kehr and Yoshitake, 2006) and FST (modified from Porsolt, 1979) experiments were carried out Sprague Dawley rats. Both rapastinel (3-30 mg/kg) and S(+)- ketamine (3-30 mg/kg) decreased immobility time in the FST when tested at 1 hour, day 2 and day 7 post-dose. S(+)-ketamine significantly elevated extracellular levels of DA, 5-HT and glutamate in the mPFC within the first 60 min post-dose. By contrast, rapastinel did not increase DA, 5-HT or glutamate levels in the mPFC at any doses tested. These data demonstrate a

distinct difference in the neurochemical basis for rapastinel's antidepressant-like activity and favourable CNS adverse effects compared to ketamine. Rapastinel's antidepressant-like activity does not require a surge of glutamate; and, increases in brain DA, 5-HT and glutamate levels by ketamine may play a significant role in its high abuse potential and psychotomimetic-like effects.

108. **M König***, B Berlin, H Lau, J Klein
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Acetylcholine levels in the brains of alpha-synuclein transgenic mice
Parkinson's disease (PD) is characterized by a loss of dopaminergic neurons in the substantia nigra pars compacta, which leads to substantial decreases of dopamine in its striatal projections. The formation of Lewy bodies in nerve cells, consisting mainly of the protein alpha-synuclein, is a typical marker for PD. However, the underlying mechanisms linking neurodegeneration, alpha-synuclein and neurotransmitter systems in PD are not well characterized.
Acetylcholine (ACh) levels in transgenic mice expressing full-length human alpha-synuclein (h-synL62) were measured using the microdialysis technique and HPLC-ECD. The mice showed strong accumulation of alpha-synuclein in striatal neurons, early onset bradykinesia and progressive motor deficits. The release of dopamine (DA) was reduced in 9-month-old transgenic mice after challenge with amphetamine. Concomitantly, basal ACh levels in this age group were higher in transgenic mice and significantly increased during stimulation with scopolamine, indicating a disinhibition of striatal cholinergic interneurons due to depletion of DA. Collectively, these results demonstrate that striatal cholinergic interneurons are affected by alpha-synuclein expression and likely contribute to the neurotransmitter dysbalance in PD.

109. **NN Malik***, M Ligocki, E Mead, G Carter, J Wolak, SN Mitchell
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Assessment of Kynurenine Pathway Metabolites as an Index of Neurodegeneration
The kynurenine pathway (KP) is a major route of tryptophan metabolism and several metabolites of this pathway have been proposed to be involved in the pathogenesis of Alzheimer's disease. Particularly, it has been shown that the KP is over activated in AD brain and that quinolinic acid accumulates in amyloid plaques. Pharmacological and genetic manipulation of the KP has also been shown to ameliorate neurodegenerative phenotypes, suggesting that it could be a viable target for the treatment of neurodegenerative disorders.
An LC MS/MS assay was developed, using esterification as well as dansylation protocols, to measure key KP metabolites, firstly in microdialysate samples, to study the role of the KP in the progression of pathology in rTg4510 mice, a transgenic mouse model of human tauopathy. In a second study, in microglia, the sensitivity to lipopolysaccharide and interferon gamma, both key activators of the KP, was measured. In both studies, the sensitivity of the KP to manipulation was also evaluated using a kynurenine inhibitor, 3-monooxygenase, an enzyme that acts as a gateway between the potentially neurotoxic and neuroprotective elements of the pathway, namely with the formation of quinolinic acid and kynurenic acid, respectively. These methods and data will be discussed.

110. **B Perez-Palomar[1,2]***, JE Ortega[1,2,3], JJ Meana[1,2,3]
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Maternal immune activation induces cortical dopaminergic hypofunction and cognitive impairments in offspring
Schizophrenia has been associated with cortical dopaminergic dysfunction. Cognitive deficits are present in schizophrenia together with classical symptoms. Prenatal exposure to infection represents a significant environmental risk factor for the development of schizophrenia. Thus, maternal immune activation (MIA) in animals produces neurochemical and behavioral abnormalities considered relevant in models of schizophrenia.
The aim was to evaluate a MIA model mimicking prenatal viral infection by the administration of poly(I:C) to pregnant dams (7.5 mg/kg ip, gestational day 9.5). Extracellular dopamine (DA) and noradrenaline (NA) concentrations were evaluated in prefrontal cortex (PFC) of adult offspring by microdialysis. In addition, cognitive impairments were also evaluated by novel-object recognition test (NORT).
Poly(I:C) mice showed decreased DA but unaltered NA, when compared with controls. Amphetamine (2.5 mg/kg ip) induced an increase of DA/NA concentrations that were lower in the poly(I:C) group, whereas the response to the NMDA receptor antagonist MK-801 (0.5 mg/kg ip) remained unaltered. Potassium-evoked release of DA/NA was not different between groups. Additionally, poly(I:C) mice showed decreased novel object discrimination in the NORT.

In conclusion, MIA induces in offspring a PFC dopaminergic hypofunction with cognitive impairment. Poly(I:C) model could be considered as a promising translational animal model for the study of schizophrenia dysfunctions.

111. **G Tanda[1]***, MCH Rohn[3], AH Newman[1], MA Coggiano[1], C Zanettini[1], JD keighron[1], JL Katz[2], L Leggio[3], MR Lee[3]
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Systemic oxytocin affects the reinforcing and neurochemical effects of methylphenidate

The hypothalamic neurohormone oxytocin and the neurotransmitter dopamine play a significant role in social bonding. Dopamine is also involved in both natural and pathologic behaviours related to reward and reinforcement, including substance use disorders. Early reports have shown that oxytocin might interfere with psychostimulant behavioural effects. Here, the effects of oxytocin were assessed in Sprague-Dawley rats: a) trained to intravenously self-administer methylphenidate (1 mg/kg, fixed ratio 5) during single daily 1-hr sessions, b) implanted with microdialysis probes to assess methylphenidate (0.1-1 mg/kg, i.v.) effects on levels of dopamine in the nucleus accumbens shell and core, and c) placed in infrared activity monitors to measure behavioural activation.

Oxytocin (0.1-2 mg/kg, i.p., 10 min before the sessions) dose-dependently decreased maximal self-administration of methylphenidate (0.03-1.0 mg/kg/injection, i.v.). Oxytocin (0.2-2 mg/kg, i.p.) had little to no significant effects on extracellular levels of dopamine in the accumbens shell, but it dose-dependently enhanced methylphenidate-stimulated (0.1-1 mg/kg, i.v.) dopamine levels in the shell (but not in the core) of the accumbens. Similar effects on methylphenidate neurochemistry were obtained when oxytocin was locally infused into the accumbens shell by reverse dialysis, suggesting these actions being mediated by its receptors in this area. Interestingly, enhancement of dopamine levels did not result in stimulation of behavioural activities at levels higher than those produced by methylphenidate alone. Voltammetry studies are ongoing to evaluate if oxytocin effects on methylphenidate are the results of changes in dopamine uptake or release.

The present results suggest that oxytocin attenuates the psychostimulant-like reinforcing effects of methylphenidate, likely acting through altered dopamine neurotransmission in the terminals of the mesolimbic system. Thus, these results confirm and extend the potential translational therapeutic value of oxytocin for psychostimulant use disorders.

Research Funded by: MDP-NIDA-IRP, NIH/DHHS; Bench-to-Bedside Grant by the OBSSR/NIH; Intramural Grant ZIA AA000218-01 (CPN Section) DICBR/NIAAA/NIH/DHHS

112. **A Thinnes***, J Klein
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Food-induced changes of acetylcholine in mouse hypothalamus

Feeding behaviour is controlled in the hypothalamus by humoral signals and afferent neuronal pathways including central cholinergic pathways. For instance, anorexigenic POMC (proopiomelanocortin) neurons receive cholinergic input. Here, we used microdialysis in wild type mice (C57Bl/6JRj) to monitor cholinergic activity in the hypothalamus. Food intake after an overnight fast increased extracellular ACh twofold in the hypothalamus. The effect lasted for about 60 minutes. Food containing no calories (kaolin pellets), or food that was presented but not accessible for the mice also increased ACh release. In contrast, injections of glucose or ß-hydroxybutyrate did not change extracellular ACh. We conclude that the increase of ACh in the hypothalamus was not caused by local detection of nutrients but by expectation of food intake, possibly involving motivational circuits in the basal forebrain.