Monitoring Molecules in Neuroscience

The 18th International Conference

June 29th – July 2nd 2022
Lyon, France

Abstracts Book

http://mmin2022.univ-lyon1.fr

mmin2022@univ-lyon1.fr

CONTENTS

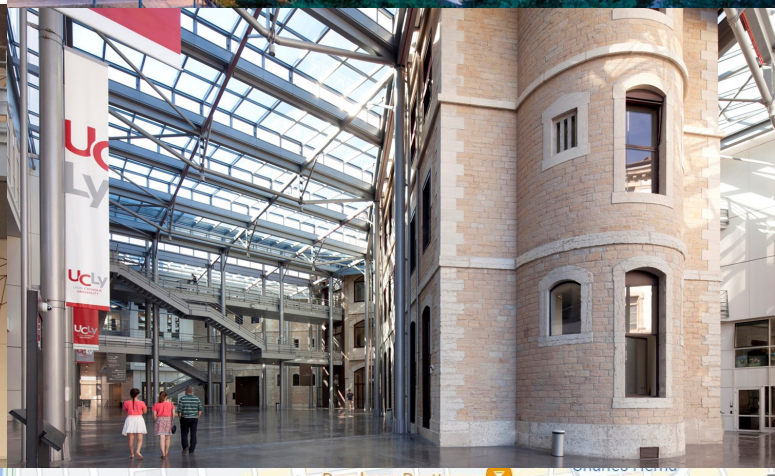
VENUE - UCLY - LYON MAP

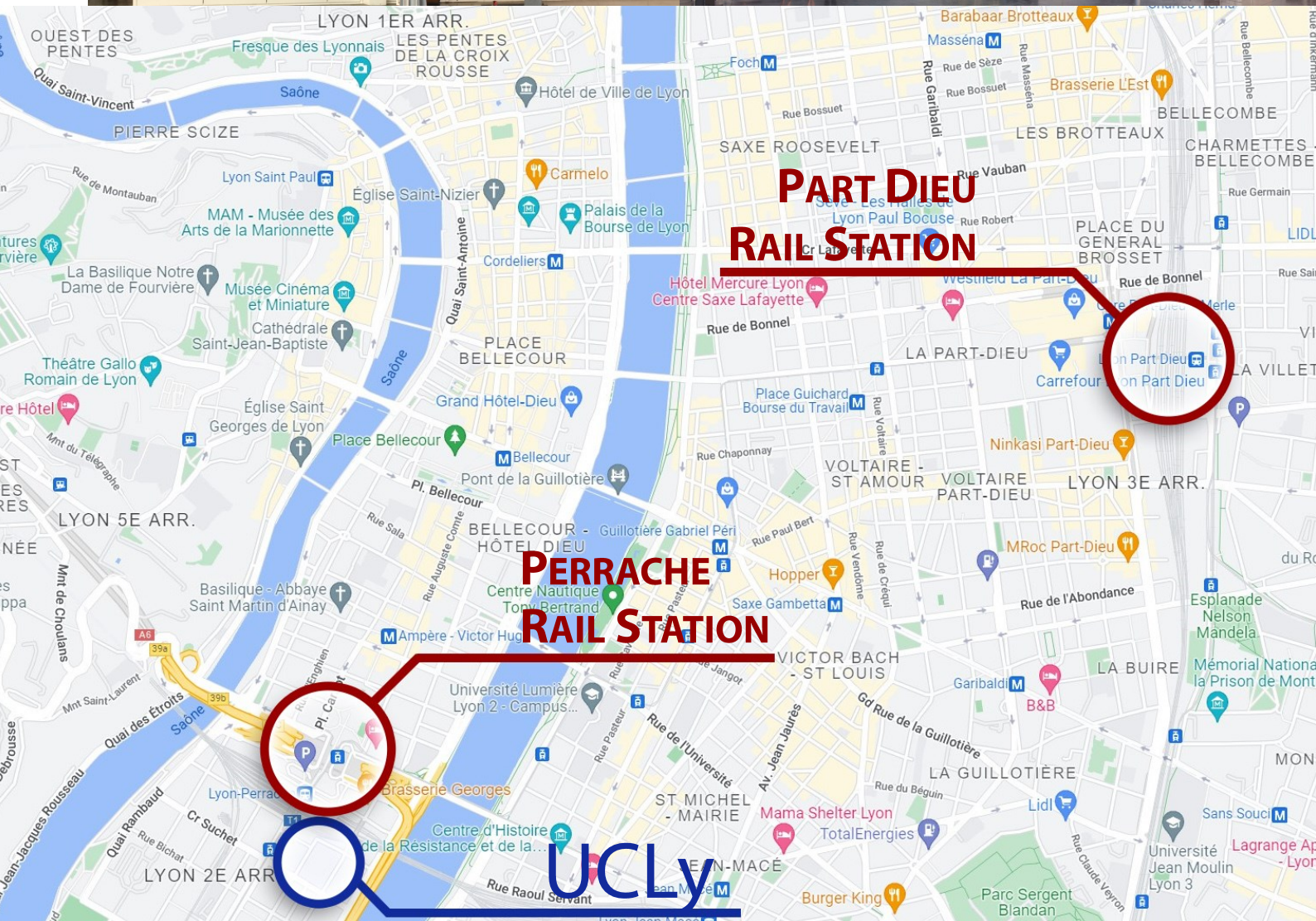
UCLY, UNIVERSITÉ CATHOLIQUE DE LYON

Campus Saint Paul

10 place des archives

69002 Lyon, France





WELCOME MESSAGE

Dear friends and colleagues,

Welcome to this 18th international conference organized in Lyon by the MMiN organizing committee. After two years of pandemic which forced us into teleworking, very often at home, this year finally allows us to present our scientific data and to interact face to face.

Thanks to all of you, Professors, researchers, engineers and students, who have devoted the time, energy and money to come from abroad to honor us with your presence for these few days dedicated to neuroscience. Congratulations to the 190 attendees, from 15 countries, that have made the effort to come!
We also give huge thanks to the companies and organizations who have generously supported the meeting and helped us to set up this wonderful event. This year exceptionally, ACS Chemical Neuroscience, Brain Sciences Journal and InTech Open editor will be awarding prizes to three talented young scientists during Friday's evening gala dinner at the Paul Bocuse restaurant. Don't miss it !

We are very happy to be able to welcome you in the magnificent premises of the Catholic University of Lyon (UCLy). [*By a strange twist of fate, part of the buildings that host our congress this year, freeing us from these two years of hypoxia, are in fact two former prisons, Saint Paul and Saint Joseph, built in 1831 and 1865, and converted by the city in 2015…]*

We are very proud to be able to present you a high-quality scientific program during these four days, and have also chosen to combine business with pleasure, in the form of French gastronomy and of Lyon cuisine in particular.
During our MMiN meeting, 4 plenary sessions, with internationally renowned speakers, will follow one another, as well as 16 symposiums held in parallel on specific subjects, all related to your fundamental and applied research interests.

The opening symposium, organized by the Paul Bocuse research Institute (IPB), will let you discover one of the research programs supported by the Institute, which is very involved in the study of the food intake control mechanisms.

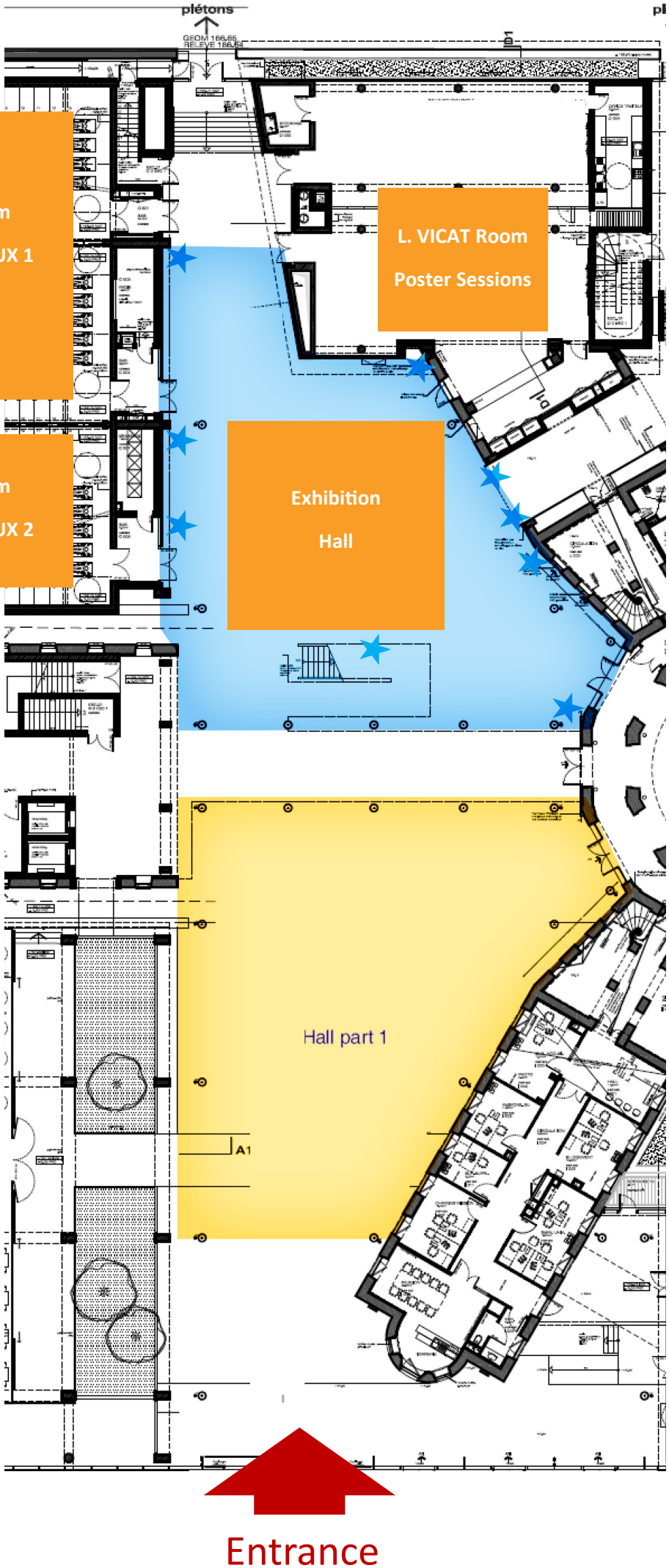
We are convinced that the MMiN meeting will be an ideal opportunity for information sharing, discussion and debate. All these face-to face interactions with your peers will certainly inspire your future projects, and we also wish you an excellent stay here in Lyon.

Barbara Ferry, co-chair

Sandrine Parrot, co-chair

On the behalf of the Local Organizing Committee

UCLY MAP


Room
MERIEUX 1
Room
MERIEUX 2
L. VICAT Room
Poster Sessions
Exhibition
Hall
Hall part 1
Entrance

PRESIDENT WELCOME MESSAGE

Welcome to MMiN Lyon, 2022. We've waited 4 years for this, our 18th international conference, the longest hiatus in our history, the reason for which requires no explanation. At long last we can learn about exciting new developments in neurochemical monitoring, and engage in lively conversations with our colleagues uninterrupted by "you're muted!" Sandrine Parrot, Barbara Ferry and their advisory committees have worked tirelessly under conditions of extreme uncertainty over the last 4 years, effectively organizing this meeting twice. Despite the hurdles faced, they have put together exceptional scientific and social programs, both of which promise a feast, for which they deserve our uttermost gratitude and respect.

Neither has the MMiN Scientific Advisory Board been twiddling their thumbs since we last met. I am pleased to report that the Society is now partnering with Podium - Conference and Association Specialists, who will help manage and expand the Society, and take much of the administrative burden from the shoulders of future Conference Chairs. Marischal DeArmond, Founder and CEO of Podium, will be mingling among us, and I encourage you to get to know him, hear what Podium has to offer, and offer him your perspective on the future direction of our Society. As a result of our partnership with Podium, we are in the closing stages of registering the Society as a Charitable Incorporated Organisation, which, among other advantages, will protect Conference Chairs from financial responsibility, and permit the Society to collect membership dues so that we may build reserves to finance our meetings and offer additional services to our members.

High on the list of priorities of such services is providing travel bursaries and poster awards for our junior scientists. Indeed, encouraging active participation of Ph.D. students and post-docs has been a hallmark of our meetings since the first meeting organized by Charles Marsden at Nottingham University in 1982. I can personally attest to this, as I presented my first poster at that meeting as a Ph.D. student in Charles' lab. I am delighted Charles is joining us in Lyon as an honoured guest of the Society and I'm sure he would like to join Sandrine, Barbara and me in extending a special welcome to the many students and post-docs among us.

Thanks to Podium, we also have a new website (and a new logo) https://monitoringmolecules.org that will host all future meetings information, and ultimately serve as a focal point for all things Monitoring Molecules in Neuroscience. The site is somewhat 'bare bones' currently but is ready to be built into the go-to place for the latest news and developments in our field, better serving and expanding our membership. But this will require all of our input, especially the aforementioned students and post-docs. So, if you would like to contribute to the development of the website please speak to me or a member of the Scientific Advisory Board at this meeting or contact Sarah-Kate Burke at Podium - mmin@podiumconferences.com

Finally, I encourage everyone to attend the Open Business Meeting on Friday when we will elect 5 new members to the Scientific Advisory Board, hear the latest news from Zoe McElligot and Sara Jones on plans for MMiN 2024 at the University of North Carolina, and choose the venue for 2026. If you are interested in running for election to the Board, please contact me, or one of the current Board members or Officers (listed below), ahead of the Business Meeting for information on the process.

Nigel T. Maidment, Ph.D.
President
International Society for Monitoring Molecules in Neuroscience
https://monitoringmolecules.org

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Monitoring Molecules in Neuroscience

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brain sciences
an Open Access Journal by MDPI

MMiN 2022 program at a glance

Start at	Wednesday June 29th	Thursday June 30th		Friday July 1st		Saturday July 2nd		Start at
8:00 AM				S7. Bringing light to the dynamics of intracellular signals in neurons (MERIEUX 1)	S8. Developments and alternatives in microdialysis (MERIEUX 2)			8:00 AM
8:30 AM		Plenary lecture by Stephanie Cragg (MERIEUX 1)						8:30 AM
9:00 AM						Plenary lecture by Sergi Ferré (MERIEUX 1)		9:00 AM
9:30 AM		*Coffee break , exhibition (HALL)*						9:30 AM
10:00 AM		S1. Monitoring neuromodulators during behavior (MERIEUX 1)	S 2. Central and peripheral control of feeding (MERIEUX 2)	*Coffee break , exhibition (HALL)*		*Coffee break , exhibition (HALL)*		10:00 AM
10:30 AM				Poster session #2** (VICAT)		S13. High temporal with high spatial resolution analysis: from synapse to vesicle (MERIEUX 1)	S14. Monitoring brain molecules by PET imaging (MERIEUX 2)	10:30 AM
11:00 AM				Plenary lecture by Ana Počivavšek (sponsored by EBBS society) (MERIEUX 1)				11:00 AM
11:30 AM								11:30 AM
12:00 PM		Lunch break, exhibition (HALL) & Poster session #1* (VICAT)		Lunch break, exhibition (HALL) & Poster session #2** (VICAT)				12:00 PM
12:30 PM	*Badge pick-up*					*Lunch break, exhibition (HALL)*		12:30 PM
1:00 PM								1:00 PM
1:30 PM	Welcome introduction (MERIEUX 1)	S3. New technologies for monitoring neuromodulators *in vivo* (MERIEUX 1)	S4. Microdialysis to assess the injured brain. (MERIEUX 2)	S9. New insights into brain purinergic signaling (MERIEUX 1)	S10. Analytical measurements in non-mammalian systems (MERIEUX 2)	Farewell Symposia		1:30 PM
2:00 PM	Opening lecture by Brigitte Kieffer (MERIEUX 1)					S15. Real-time measurement of neurotransmitters and intracellular signaling *in vivo* (MERIEUX 1)	S16. Analytical Chemistry Symposium / Novel frontiers in neurochemical analysis (MERIEUX 2)	2:00 PM
2:30 PM								2:30 PM
3:00 PM	*Coffee break (HALL)*			*Coffee break , exhibition (HALL)*				3:00 PM
3:15 PM								
3:30 PM		*Coffee break , exhibition (HALL)*				Closing (MERIEUX 1)		3:30 PM
4:00 PM				S11. From the inside out - dopamine neuron function and dysfunction (MERIEUX 1)	S12. Optical techniques to probe function deeper in the brain (MERIEUX 2)			
4:30 PM	"Paul Bocuse Institute" Opening Symposium (MERIEUX 1)	S5. Investigate astrocyte functions by monitoring dynamic change in glioactive molecules (MERIEUX 1)	S6. Functional and anatomical investigations of valence coding (MERIEUX 2)					
5:00 PM								
5:15 PM								
5:30 PM				Open meeting (MERIEUX 1)				
6:00 PM		Close meeting		Transfer to Bocuse restaurant				
6:15 PM								
6:30 PM	Special tasting buffet by Elior and the Research Institute Paul Bocuse (HALL) + Welcome reception (VICAT)							
7:00 PM				Visit & Gala dinner (Bocuse Abbaye)				
8:00 PM		** odd-numbered posters*						
9:00 PM		*** even-numbered posters*						
10:00 PM								
11:00 PM				Transfer back to Lyon				

WEDNESDAY JUNE 29TH - AFTERNOON

Room MERIEUX 1

12:30	Registration	
1:30	**Welcome introduction**	
2:00	Opening lecture by **Brigitte Kieffer** *Chair:* **Nigel Maidment** INSERM & University of Strasbourg France Genetic tools to study G protein coupled receptors *in vivo*	**L1**
3:00	*Coffee break*	**HALL**
	"Paul Bocuse Institute" Opening Symposium	
3:30	**Marlou Lasschuijt** Wageningen University, The Netherlands Through the senses: Exploring how chemosensory signals drive and inhibit food intake	**OS1**
4:20	**Marc Tittgemeyer** Max Planck Institute for Metabolism Research, Germany Translating physiological need to motivated behaviour – Metabolic signals as modulators of eating behaviour	**OS2**
5:10	**Anestis Dougkas** Institut Paul Bocuse Research Centre, France Sensory alteration in cancer patients during chemotherapy and its relation to food behavior	**OS3**
6:00	Special tasting buffet by the Research Institute Paul Bocuse Welcome reception	**HALL** *(until 8:30PM)*

Room VICAT

THURSDAY JUNE 30TH - MORNING

8:30	Plenary lecture by **Stephanie Cragg** *Chair:* **Paul E.M. Phillips** University of Oxford, UK **L2** Axonal gating of striatal dopamine transmission by diverse stratial neuronal and non-neuronal networks	Room MERIEUX 1
9:30	*Coffee break/exhibition* **HALL**	
	S1 Monitoring neuromodulators during behavior using old and new methods: Differences and new opportunities *Co-chairs:* **Eleanor H. Simpson, Ali Mohebi**	Room MERIEUX 1
10:00	**Mark Walton** University of Oxford, UK **S1C1** Tracking striatal dopamine over time for reward	
10:25	**Armando Salinas** National Institute on Alcohol Abuse and Alcoholism, USA **S1C2** Striatal dopamine dynamics assessed with fast-scan cyclic voltammetry and dLight	
10:50	**Dan Covey** Lovelace Biomedical, USA **S1C3** Motivation and accumbal dopamine release operate in a valence- and cost-dependent manner	
11:15	**Ali Mohebi** UCSF, USA **S1C4** Neuromodulation of forebrain dynamics during cognitively demanding tasks	
11:40	**Patrick R. Melugin** Vanderbilt Brain Institute, USA **S1C5** Deciphering the role of dopamine in the mouse medial prefrontal cortex	
	S2 Central and peripheral control of feeding *Co-chairs:* **Fabien Naneix, Yvan Peterschmitt**	Room MERIEUX 2
10:00	**Daniela Cota** French Institute of Health Inserm, France **S2C6** Hypothalamic bile acids-TGR5 signaling: a new player in energy balance regulation	
10:25	**Giuseppe Gangarossa** Université de Paris, France **S2C7** Exploring gut-brain circuits to understand homeostatic and reward adaptations in eating disorders	
10:50	**Pierre-Yves Risold** Université de Franche-Comté, France **S2C8** The PSTN in feeding behavior	
11:15	**Stephanie Borgland** University of Calgary, Canada **S2C9** Obesity induced disinhibition of the orbitofrontal cortex leads to impairement in goal-directed behaviour	
11:40	**Fabien Naneix** University of Aberdeen, UK **S2C10** Age-dependent effects of protein restriction on dopamine release	
12:00	*Lunch break/exhibition* **HALL** **& Poster session #1** *(odd-numbered posters)* **Room VICAT**	

THURSDAY JUNE 30TH - AFTERNOON

S3 New technologies for monitoring neuromodulators *in vivo*

Co-chairs: **Tommaso Patriarchi, Robert Kennedy**

Room MERIEUX 1

1:30 **Robert T. Kennedy** University of Michigan, USA **S3C11**
Deep chemical analysis of microdialysate by LC-MS: in vivo metabolomics

1:55 **Tommaso Patriarchi** University of Zurich, Switzerland **S3C12**
High-resolution imaging of neuromodulators using genetically encoded fluorescent sensors

2:20 **Alan Jasanoff** Massachusetts Institute of Technology, USA **S3C13**
Functional and molecular MRI of dopaminergic circuitry in the rodent brain

2:45 **Sara R. Jones** Wake Forest University School of Medecine, USA **S3C14**
Stress- and ethanol-induced changes in striatal extracellular levels of dynorphin as measured by microdialysis in mice

3:10 **Nako Nakatsuka** University of Zurich, Switzerland **S3C15**
Nanoscale aptamer-modified biosensors monitor dopamine and serotonin ex vivo

S4 Microdialysis to assess the injured brain

Co-chairs: **Martyn Boutelle, Adrian Michael**

Room MERIEUX 2

Raimund Helbok Medical University of Innsbruck, Austria **S4C16**
Neurochemical monitoring of the brain - the neurointensive care perspective

Adrian Michael University of Pittsburgh, USA **S4C17**
Microdialysis in the injured brain

Keri Carpenter University of Cambridge, UK **S4C18**
Microdialysis monitoring of cerebral metabolism in TBI

Martyn Boutelle Imperial College London, UK **S4C19**
New technologies for real-time patient monitoring using microdialysis

Chiara Cicatiello Imperial College London, UK **S4C20**
Wireless electrochemical device for real-time monitoring of neural ionic changes in patients with severe traumatic brain injury

3:30 *Coffee break/exhibition* **HALL**

S5 Investigate astrocyte functions by monitoring dynamic change in glioactive molecule

Co-chairs: **Pierre Marquet, Jean-Marie Petit**

Room MERIEUX 1

4:00 **Armelle Rancillac** Collège de France, France **S5C21**
Real time measurment of adenosine in the ventrolateral proptic nucleus (VLPO): a crucial hypnogenic molecule

4:25 **Jean-Yves Chatton** University of Lausanne, Switzerland **S5C22**
Imaging tools for extracellular potassium and lactate in brain tissue

4:50 **Pierre Marquet** CERVO Brain Research Center/Laval University, Canada **S5C23**
Quantitative phase-digital holographic microscopy to explore astrocyte functional phenotypes

5:15 **Nasser Haddjeri** Stem cell and Brain Research Institute, France **S5C24**
Study of the astroglial control of the antidepressant response with pharmacological and chemogenetic approaches

5:40 **Kevin Richetin** CHUV-UNIL, Switzerland **S5C25**
Neuron-derived extracellular vesicles containing tau disrupt astrocytes function and memory performance

S6 Functional and anatomical investigations of valence coding

Co-chairs: **James McCutcheon, Anna Beyeler**

Room MERIEUX 2

Amber Alhadeff University of Pennsylvania, USA **S6C26**
Gut-brain signaling and the control of food intake

Anna Beyeler French Institute of Health Inserm, France **S6C27**
Role of anterior insula circuits in emotional valence and anxiety

Ingo Willuhn Netherlands Institute for Neuroscience, The Netherlands **S6C28**
A unidirectional but not uniform striatal landscape of dopamine signaling for motivational stimuli

Ream Al-Hasani Washington University in St. Louis, USA **S6C29**
Differential role of accumbal opioid peptides in food reward and stress

Kenneth Kishida Wake Forest School of Medicine, USA **S6C30**
Sub-second fluctuations of extracellular dopamine in humans encode valence-partitioned reward and punishment prediction errors

6:00 **Close meeting**

FRIDAY JULY 1ST - MORNING

S7 Bringing light to the dynamics of intracellular signals in neurons

Co-chairs: **Pierre Vincent, Oliver Griesbeck**

Room MERIEUX 1

8:00 **Oliver Griesbeck** Max-Planck-Institute for Biological Intelligence, Germany **S7C31**
New fluorescent biosensors for interstitial calcium

8:25 **Julie Perroy** CNRS Institut de Génomique Fonctionnelle, France **S7C32**
BRET biosensors for live imaging of ERK and mTOR signaling pathways activation in neuronal plasticity *in vivo*

8:50 **Liliana Castro** Sorbonne Université, France **S7C33**
Biosensor imaging to reveal the specificities of cAMP/PKA signal integration in the striatum

9:15 **Julien Courchet** French Institute of Health Inserm, France **S7C34**
Control of cortical axon morphogenesis through mitochondria trafficking and function

9:40 **Sebastian Kruss** Ruhr-University Bochum, Germany **S7C35**
A near infrared fluorescent sensor paint to image dopamine signaling

S8 Developments and alternatives in microdialysis

Co-chairs: **Anne Andrews, Stephen Weber**

Room MERIEUX 2

Anne Andrews UCLA, USA **S8C36**
Microdialysis and beyond for multiplexed neurochemical monitoring

Tod Kippin UCSB, USA **S8C37**
Electrochemical aptamer-based biosensors for pharmacology and neuroscience

Florie Le Prieult AbbVie Deutschland GmbH & Co. KG, Germany **S8C38**
A window to the brain: on-line measurement of biologics concentrations and target engagement using microdialysis and cOFM

Stephen Weber University of Pittsburgh, USA **S8C39**
A microfluidic sampling device based on two-photon polymerization for investigating processes in brain

Vagif Abdulla University of Connecticut, USA **S8C40**
Wireless, battery-free push-pull microsystem for membrane-free neurochemical sampling in freely moving animals

10:00 *Coffee break/exhibitors* **HALL**

10:30 **Poster session #2** *(even-numbered posters)* **Room VICAT**

11:00 Plenary lecture by **Ana Počivavšek** *Chair:* **Zoe A. McElligott**
University of South Carolina, USA **L3**
Sleep, cognition, and kynurenic acid: building blocks of mental health
sponsored by EBBS society

Room MERIEUX 1

12:00 *Lunch break/exhibition* **HALL**

& Poster session #2 *(even-numbered posters)* **Room VICAT**

FRIDAY JULY 1ST - AFTERNOON

S9 New insights into brain purinergic signaling

Co-chairs: **Jill Venton, David Blum**

Room MERIEUX 1

1:00 **Rodrigo Cunha** University of Coimbra, Portugal **S9C41**
Increased ATP release, ATP-derived adenosine formation and adenosine A2A receptor density as determinants of brain dysfunction

1:25 **Francisco Ciruela** Universitat de Barcelona, Spain **S9C42**
Adenosine receptor heteromers: biasing antipsychotics

1:50 **Colby Witt** University of Cincinnati, USA **S9C43**
Developing tools to measure dynamic guanosine signaling in the brain

2:15 **Jill Venton** University of Virginia, USA **S9C44**
Regulation of spontaneous and mechanosensitive adenosine release

2:40 **David Blum** French Institute of Health Inserm, France **S9C45**
Impact of hippocampal upregulation of adenosine A2A receptors in the mouse hippocampus

S10 Analytical measurements in non-mammalian systems

Co-chairs: **Michael Johnson, Laurent Seugnet**

Room MERIEUX 2

1:00 **Michael Johnson** University of Kansas, USA **S10C46**
Expanding neurochemical measurements in zebrafish

1:25 **Laurent Seugnet** Lyon Neuroscience Research Center, France **S10C47**
LAT1 dependent amino-acid transport and sleep/wake regulation, Iinvestigation using HPLC in Drosophila

1:50 **Andrew Ewing** University of Gothenburg, Sweden **S10C48**
Intracellular electrochemistry in drosophila shows exocytosis is partial and complex

2:15 **Pierre-Yves Plaçais** CNRS, PSL Research University, France **S10C49**
Metabolic control of memory formation revealed by 2-photon imaging of brain energy metabolism in Drosophila

2:40 **Pelumi Obasaju** University of Leicester, UK **S10C50**
Optimising fast scan cyclic voltammetry analysis for the detection of dopamine in the zebrafish brain

3:00 *Coffee break/exhibition* **HALL**

S11 From the inside out - dopamine neuron function and dysfunction

Co-chairs: **Leslie Sombers, Jyoti C. Pate**

Room MERIEUX 1

3:15 **Margaret Rice** New York University Grossman School of Medicine, USA **S11C51**
Regulation of somatodendritic dopamine release from the inside

3:40 **Jyotiben C. Patel** New York University Grossman School of Medicine, USA **S11C52**
Talking to one's self: autoregulation of striatal dopamine release by co-released gaba

4:05 **Katherine Brimblecombe** University of Oxford, UK **S11C53**
Can we utilise enogenous regulators of L-TYPE channels as neuroprotective strategies against Parkinson's

4:30 **Leslie A. Sombers** NC State University, USA **S11C54**
Striatal dopamine and hydrogen peroxide transients associated with L-DOPA included rotation in hemiparkinsonian rats

4:50 **Zahra Farahbakhsh** Vanderbilt University, USA **S11C55**
Long-lasting alterations in axonal dopamine release regulation & upstream transcription induced by ethanol drinking in macaques

S12 Optical techniques to probe function deeper in the brain

Co-chairs: **Paul Slesinger, Zhenpeng Qin**

Room MERIEUX 2

3:15 **Paul Slesinger** Mount Sinai School of Medecine, USA **S12C56**
Probing striatal circuits with photo-releasable azosomes

3:40 **Guosong Hong** Stanford University, USA **S12C57**
Seeing the sound: optical and ultrasonic interfaces for neuromodulation

4:05 **Markita Landry** University of California Berkeley, USA **S12C58**
Imaging neuromodulators in the brain with near-infrared fluorescent nanosensors

4:30 **Zhenpeng Qin** University of Texas at Dallas, USA **S12C59**
Probing neuropeptide volume transmission *in vivo* by simultaneous near-infrared light release and optical sensing

4:50 **Anistasha Lightning** Lyon Neuroscience Research Center, France **S12C60**
Light stimulation reduction in neuronal activity is dependent on the temporal pattern and light power used

5:15 **Open meeting** **Room MERIEUX 1**

6:15 *Transfer to restaurant* **GALA Dinner - Bocuse Abbaye**

SATURDAY JULY 2ND - MORNING

9:00	Plenary lecture by **Sergi Ferré** — *Chair:* **David Blum** National Institute on Drug Abuse, NIH, USA — **L4** A journey with *in vivo* microdialysis	Room MERIEUX 1
10:00	*Coffee break/exhibition*	**HALL**

	S13 High temporal with high spatial resolution analysis: from synapse to vesicle *Co-chairs:* **Andrew Ewing, Nhu Phan**	Room MERIEUX 1	**S14 Monitoring brain molecules by PET imaging** *Co-chairs:* **Luc Zimmer, Nicolas Costes**	Room MERIEUX 2
10:30	**Silvio Rizzoli** University Medical Center Göttingen, Germany **S13C61** Integrated nanoSIMS and fluorescence microscopy analysis of neuronal cells		**Luc Zimmer** Université Claude Bernard Lyon 1, France **S14C66** How PET imaging is advancing the discovery of new CNS drugs	
10:55	**Christian Amatore** Ecole Normale Supérieure, France **S13C62** Quantitative nano-amperometric measurement and of sub-quantal glutamate release by living neurons		**Thierry Billard** CNRS, France **S14C67** Design and radiolabeling strategies for brain PET radiotracers	
11:20	**Nhu Phan** University of Gothenburg, Sweden **S13C63** Imaging of lipids and proteins in nerve cells with sims - from non-targeted to targeted bioimaging		**Léon Tremblay** Institut des Sciences Cognitives, France **S14C68** PET imaging exploration dopamine and serotonin transmissions in psychiatric disorders of Parkinson's disease	
11:45	**Martina Damenti** KTH, Royal Institute of Technology - Scilifelab, Sweden **S13C64** Clusters or condensates? How arc regulates AMPA receptors level		**Eric Salmon** University of Liege, Belgium **S14C69** PET imaging contributions to the understanding of neurodegenerative pathologies	
12:10	**Stefania Rabasco** University of Gothenburg, Sweden **S13C65** Combining tem and nanosims imaging to discern vesicle compartments in PC12 cells and quantify isotopic dopamine concentration		**Sarah Chaib** Hospices Civils de Lyon, France **S14C70** Preclinical PET/fMRI imaging of a biased agonist of serotonin receptors in a rat model of L-DOPA-induced dyskinesia	

12:30	*Lunch break/exhibition*	**HALL**

SATURDAY JULY 2ND - AFTERNOON

Farewell Symposia

S15 Real-time measurement of neurotransmitters and intracellular signaling *in vivo* with newly developed genetically-encoded sensors
Co-chairs: **David Lovinger, Paul Phillips, Armando Salinas**

Room MERIEUX 1

1:30 **David Lovinger** National Institutes of Health, USA **S15C71**
Dynamics and modulation of striatal acetylcholine

1:55 **Tianyi Mao** OHSU, USA **S15C72**
Imaging signaling events in respone to neuromodulators at single cell resolution *in vivo*

2:20 **Guohong Cui** National Institutes of Health, USA **S15C73**
Local neural mechanisms of therapeutic deep brain stimulation in Parkinson's disease

2:45 **Huaibin Cai** National Institute on Aging, NIH, USA **S15C74**
Deficiency in endocannabinoid synthase daglb contributes to Parkinson's disease and dopaminergic neuron dysfunction

3:10 **James McCutcheon** UiT The Arctic University, Norway **S15C75**
Nutrient-specific appetites revealed by *in vivo* fibre photometry

S16 Analytical Chemistry Symposium / Novel frontiers in neurochemical analysis: Complementary tools providing fundamentally novel perspectives on the brain function
Co-chairs: **Parastoo Hashemi, Stephane Marinesco**

Room MERIEUX 2

Stéphane Marinesco French Institute of Health Inserm, France **S16C76**
Brain glycogen stores recruitment during spreading depolarizations evidenced by microelectrode biosensors

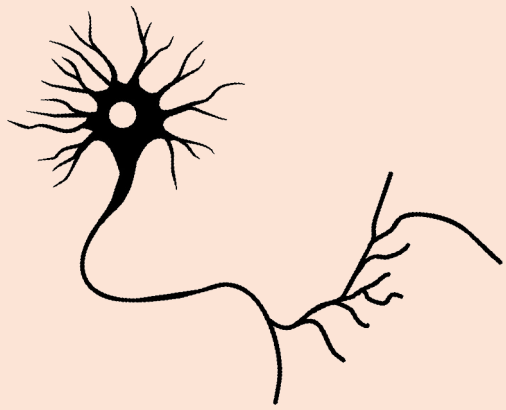
Louis Eric Trudeau Université de Montréal, Canada **S16C77**
Exploring surface-enhanced raman scattering optophysiology for making sense of complex brain neurochemistry

Jonathan Marvin HHMI - Janelia Research Campus, USA **S16C78**
Recent developments in intensity-based neurotransmitter and metabolite-sensing fluorescent reporters

Marsilea Harrison Nutromics Ltd., Australia & Imperial College London, UK **S16C79**
Fiber-based biosensors and microfluidics for monitoring transient neurochemicals

Parastoo Hashemi Imperial College London, UK **S16C80**
Novel frontiers in fast voltammetry: new analytes and models

3:35 **Closing** **Room MERIEUX 1**



Plenary lectures

GENETIC TOOLS TO STUDY G PROTEIN COUPLED RECEPTORS IN VIVO

Brigitte L Kieffer

CRBS, INSERM U1114, Strasbourg France

Keywords : G protein-coupled receptor ; opioid receptor ; addiction ; GPR88 receptor ; neuropsychiatric disorders

Revolutions in G protein-coupled receptor (GPCR) research and fascinating developments in basic neuroscience have tremendously advanced our knowledge on brain function, and opened new avenues to treat brain disease. This presentation will discuss how new genetic mouse tools have allowed deciphering opioid receptor localization and function in the rapidly evolving area of opioid research, and in particular how opioid receptors operate within the neurocircuitry of addiction. Recent work linking mu opioid receptor gene and drug activities to whole-brain functional networks, using by fMRI in mice, will also be presented. Finally, we will present how similar approaches have allowed progress in the study of GPR88, a fascinating orphan receptor considered a promising target to treat neuropsychiatric disorders,

AXONAL GATING OF STRIATAL DOPAMINE TRANSMISSION BY DIVERSE STRIATAL NEURONAL AND NON-NEURONAL NETWORKS

Stephanie Cragg and research group

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Keywords: dopamine, voltammetry, fluorescent neurotransmitter sensors, calcium imaging, voltage imaging, astrocytes

Dopamine in the mammalian striatum plays critical roles in motivation, action selection and reinforcement learning. In turn, dysregulation of dopamine signalling underlies behavioural dysfunction in multiple psychomotor disorders that include Parkinson's disease and addictions. Midbrain dopamine neurons in the substantia nigra and ventral tegmental area display action potentials at a range of instantaneous frequencies, but whether and how this dynamic activity is relayed into the release of dopamine from their exuberantly branched axonal arbours in striatum will be governed by processes operating locally within these axons.
We have explored how a range of local striatal circuits as well as intrinsic mechanisms operating on dopamine axons govern dopamine release in *ex vivo* mouse striatum. Through use of a range of methods for real-time monitoring of neuromodulators and activity (detection of dopamine release with fast-scan cyclic voltammetry, imaging axonal activity using genetically encoded reporters of calcium and voltage, striatal electrophysiological recordings, and imaging of genetically encoded fluorescent neurotransmitter sensors), we have recently elucidated that dopamine release is under the control of several neuromodulators (including ACh, GABA, adenosine), and that short-term plasticity in dopamine release is regulated by a non-canonical role for the dopamine transporter. Furthermore, looking beyond neuronal networks to non-neuronal cells, we have identified multiple mechanisms through which dopamine transmission is regulated by striatal astrocytes, through setting the tone of key axonal neuromodulators. I will highlight some of our recent findings from a range of approaches to monitor neuromodulation that illustrate how diverse processes in striatum powerfully filter dopamine output from dopamine axons.

SLEEP, COGNITION, AND KYNURENIC ACID: BUILDING BLOCKS OF MENTAL HEALTH

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University of South Carolina School of Medicine, Columbia, South Carolina

Keywords : sleep, wake, schizophrenia, astrocyte, neurodevelopment

Dysfunction in tryptophan metabolism via the kynurenine pathway (KP) has been implicated in the pathology of neurodevelopmental and psychiatric disorders, including schizophrenia (SZ) and bipolar disorder. Astrocyte-derived KP metabolite kynurenic acid (KYNA) is elevated in the brain of individuals with these severe psychiatric illnesses and has been linked to cognitive impairments in patients. Modest increases in KYNA, which modulate cholinergic and glutamatergic neurotransmission, may contribute to sleep disturbances (Pocivavsek et al. *Sleep* 2017), a common complaint among patients suffering from psychotic disorders that can exacerbate cognitive problems. We are currently investigating the novel hypothesis that KYNA represents a key molecular link between sleep disturbances and cognitive dysfunction. Based on the etiology that neurodevelopmental risk factors including maternal immune activation, stress, and obstetric complications result in an increase in tryptophan metabolism to kynurenine, we developed an experimental system in rodents where brain KYNA is elevated during the last week of embryonic development ("EKyn paradigm") by feeding dams chow laced with the direct bioprecursor kynurenine. We have studied long-lasting impacts in adult EKyn offspring of both sexes including dysfunctions in a) learning and memory, b) sleep and arousal behaviors with polysomnographic recordings that combine electroencephalogram (EEG) and electromyogram (EMG) in rodents, c) biochemical dynamics of KP metabolites, d) extracellular neurotransmitters levels in the brain, and e) genomic rhythmicity. Taken together, we have determined conspicuous sex differences whereby overall male EKyn offspring are more adversely impacted by prenatal KYNA elevation. Our ongoing and future studies highlight translational therapeutic strategies, focusing on inhibiting kynurenine aminotransferase II (KAT II) as a mechanistic avenue to reduce KYNA and improve outcome for individuals suffering from neurodevelopmental and psychiatric disorders.

A JOURNEY WITH *IN VIVO* MICRODIALYSIS

Sergi Ferré

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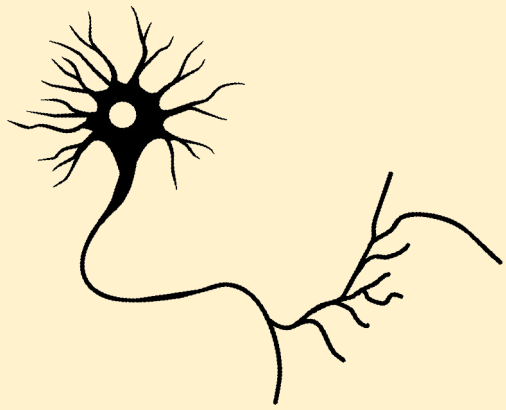
Keywords: In vivo microdialysis, G protein-coupled receptor heteromers, striatum, ventral tegmental area

I review the key role than i*n vivo* microdialysis has played in the elucidation of the functional significance of G protein-coupled receptor heteromers. It started in the nineties, with the pioneer application of the dual-probe microdialysis approach in the laboratory of Urban Ungerstedt. Those experiments allowed to demonstrate the ability of specifically interacting ligands of adenosine A_{2A} receptors (A_{2A}Rs) and dopamine D_2 receptors (D_2Rs) and ligands of A_1 receptors (A_1Rs) and D_1 receptors (D_1Rs) in the differential functional modulation of the two subtypes of striatal GABAergic efferent neurons. This was followed by a series of studies which indicated that those pharmacological interactions were largely mediated by A_{2A}R-D_2R and A_1R-D_2R heteromers, which have become targets for the treatment of Parkinson's disease and other basal ganglia disorders. Application of different adenosine receptor ligands in the striatum by reverse dialysis led to the discovery of A_1R-A_{2A}R heteromers in cortico-striatal glutamatergic terminals and to their role in the adenosine-mediated modulation of basal and electrically or optogenetically-induced glutamate release. The optogenetic experiments implied the development of a modified microdialysis probe, with an embedded optic fiber. The same technique allowed demonstrating a significant role of dopamine D_4R, which forms heteromers with D_2R, in the dopamine-mediated modulation of cortico-striatal glutamate release. Both striatal A_1R-A_{2A}R and D_2R-D_4R heteromers represent significant targets for the treatment of restless legs syndrome. Another modified microdialysis probe, with an embedded microinfusion fiber, was also develop in our laboratory to study the role of neuropeptide and hormone receptor heteromers localized in the ventral tegmental area in the modulation of dopaminergic neuronal function. This technique also allowed the local application of synthetic peptides with the ability to disrupt specific receptor heteromers, which recently provided the *in vivo* demonstration of heteromers of μ-opioid receptors (MORs) and galanin Gal_1 receptors (Gal_1Rs) and their significant involvement in the dopaminergic effects of opioids.



Communications

Opening Symposium

THROUGH THE SENSES: EXPLORING HOW CHEMOSENSORY SIGNALS DRIVE AND INHIBIT FOOD INTAKE

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Wageningen university and Research, department of Human Nutrition and Health, Sensory Science and Eating Behaviour Group

Keywords : Food intake, Satiation, Neurophysiological mechanisms

Sensory perception of foods determines our preference for food products which ultimately shapes our diet. Besides food preference, sensory signals impact our metabolic responses directly via the brainstem or through processing in higher regions of the brain. Additionally, food sensory properties such as food texture affect eating behaviour and digestion and consequent metabolic responses, such as glucose and insulin responses. Summarized, there seem to be two main mechanisms through which sensory signals affect food intake; 1) a direct effect of sensory signals on satiation through attenuated hedonic reactions to the food's flavour, inducing sensory satiation and 2) an indirect effect of sensory properties affecting digestion and metabolic responses. A better understanding of how food sensory properties affect food intake could provide the basis for public health guidelines on eating behaviour.

TRANSLATING PHYSIOLOGICAL NEED TO MOTIVATED BEHAVIOUR – METABOLIC SIGNALS AS MODULATORS OF EATING BEHAVIOUR

Marc Tittgemeyer

Max Planck Institute for Metabolism Research, Cologne, Germany

Keywords: motivated behaviour, dopamine function,chemical senses, sensory learning, nutrition

Through learning processes, sensory signals gain a motivational force and enable the brain to direct actions and adapt our behaviour to maintain physiological needs. To that end, sensory computations in the brain must be regulated by the body's momentary metabolic state, taking into account external sensory cues. The talk will discuss physiological pathways by which bodily metabolic signals are communicated through the brain to interact with sensory perception and learning processes to guide motivated behaviour. A particular emphasis will be on putative mechanisms for how the brain transforms energetic signals into the desire to eat and on the question how signals that convey nutritional information from the periphery to the brain regulate food reinforcement and choice.

SENSORY ALTERATION IN CANCER PATIENTS DURING CHEMOTHERAPY AND ITS RELATION TO FOOD BEHAVIOR

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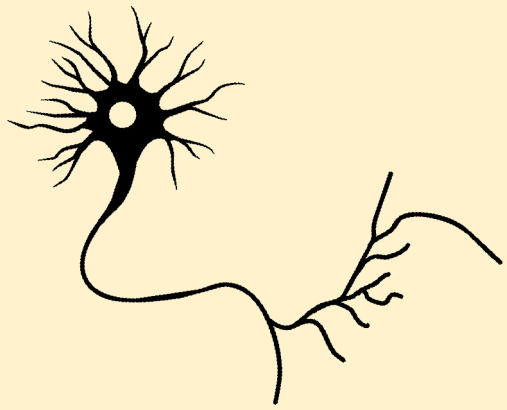
Keywords: Food, Nutrition, Taste, Pleasure of meals, Cancer, Chemotherapy

Chemotherapy treatments can induce various undesirable effects, including sensory disturbances leading to a change in food preferences that can result in a significant reduction in the pleasure of eating and food intake. The aim of the presented work conducted as part of the PhD thesis of Kenza Drareni, is to explore the effect of chemotherapy during cancer on olfactory and gustatory abilities of patients, and the consequences that this may have on their eating behavior. The first part of this work focuses on understanding the variability of sensory changes and their consequences on patients' eating behavior. Our results highlighted three main sensory profiles: patients with no sensory impairment, patients with hyposensitivity, and patients with hypersensitivity to olfactory / gustatory stimuli. Patients with impaired olfactory / gustatory abilities expressed also changes in their food behavior. The classification of patients based on their self-reported sensory abilities highlighted the negative impact of hyposensitivity on food taste perception. The classification based on psychophysical assessment of olfactory abilities showed a change in consumption habits in patients with hyposmia. Both approaches found a general downward trend in perceptual abilities of cancer patients treated with chemotherapy. In the second part of this work, we examined the effect of food sensory enhancement as a coping strategy to sensory alterations. The results suggest that taste or aroma enhancement increases food liking in patients with decreased olfactory/ taste sensitivity, and patients who did not report taste and smell deficits, but it has no effect on the hedonic rating of food in the group of control subjects. This work highlights the inter-individual diversity existing between patients and confirms the involvement of olfactory / taste alterations in patients' food behavior modification. Our results stress the importance of personalized nutritional management of patients considering their sensory alteration profile.



Communications

TRACKING STRIATAL DOPAMINE OVER TIME FOR REWARD

Mark Walton[1,2]

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Keywords : Dopamine, voltammetry, sensors, reward

I have been interested in methods for monitoring dopamine release during complex behaviours for many years. Over this period, my group has developed from exclusively using fast-scan cyclic voltammetry to monitor dopamine release to our current lab setup where the majority of our work now makes use of genetically-encoded neurotransmitter sensors. I will discuss how that has changed the types of scientific questions we can ask and analyses we can perform, with examples for some of our recent work recording dopamine receptor binding with fluorescent sensors in mice performing a multi-step decision making task that suggests striatal dopamine signals can carry separate information about reward prediction errors, movement and local reward rates at different timescales.

STRIATAL DOPAMINE DYNAMICS ASSESSED WITH FAST-SCAN CYCLIC VOLTAMMETRY AND DLIGHT

Armando G. Salinas[1,2,7], Jeong O. Lee[1], Shana M. Augustin[1,8], Shiliang Zhang[3], Tommaso Patriarchi[4,5], Lin Tian[4], Marisela Morales[6], Yolanda Mateo[1], David M. Lovinger[1]

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Keywords : photometry, biosensor, cocaine, Pavlovian

Fast-scan cyclic voltammetry (FSCV) is an electrochemical method used to detect dopamine on a subsecond time scale. Recordings using FSCV in freely behaving animals revolutionized the study of behaviors associated with motivation and learning. Despite this advance, FSCV cannot distinguish between catecholamines, which limits its use to brain regions where dopamine is the predominant neurotransmitter. It has also been difficult to detect dopamine *in vivo* in some striatal subregions with FSCV. Recently, fluorescent biosensors for dopamine were developed, allowing for discrimination between catecholamines. However, the performance of these biosensors relative to FSCV has not been determined. Thus, we compared fluorescent photometry responses of the dopamine biosensor, dLight, with FSCV. We also used dLight photometry to assess changes in tonic and phasic dopamine, which has not been possible with FSCV. Finally, we examined dopamine dynamics during Pavlovian conditioning in striatal subregions, including the dorsolateral striatum where dopamine measurements are challenging with FSCV.

MOTIVATION AND ACCUMBAL DOPAMINE RELEASE OPERATE IN A VALENCE- AND COST-DEPENDENT MANNER

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Keywords: Dopamine, Nucleus accumbens, Fiber photometery, Fluorescence, Motivation

A desire to seek out rewarding stimuli and avoid potential harms is necessary for survival and disruptions in valence-based decisions are a common feature of many neuropsychiatric and neurological disorders. Dopamine (DA) release in the nucleus accumbens (NAc) controls the propensity to overcome effortful costs during reward seeking, yet how accumbal DA release motivates active avoidance is unclear. Our prior work using fast-scan cyclic voltammetry (FSCV) shows that NAc DA release diametrically responds to cues signaling increasing cost of reward, decreasing or increasing in response to cues signaling trial onset or offset, respectively, as a function of escalating reward costs. Here, we use a fluorescent DA sensor, dLight1.3, and fiber photometry recordings to differentiate accumbal DA dynamics during positive (sucrose) and negative (foot shock) reinforcement. We find that NAc DA release diametrically responds to cues and reinforcers in a valence-dependent manner, increasing to reward -predictive cues/successful avoidance and decreasing to avoidance-predictive cues/reward receipt. Moreover, mice display striking differences in their willingness to exert effort according to valence, such that mice are much more sensitive to increasing costs (i.e., response ratio) when lever pressing to avoid shocks versus earn sucrose reward. DA release scales in magnitude according to exerted effort during reward-seeking tasks, as in our prior work, but does not reflect effort during shock-avoidance tasks. However, when given the choice, mice preferentially respond to avoid shock versus earn sucrose reward if response cost is low, and the cue-evoked DA response predicts subsequent choice. This work highlights the difficulty in defining motivation based on a single variable (effort versus choice) and in describing DA function based on positive or negative valence alone.

NEUROMODULATION OF FOREBRAIN DYNAMICS DURING COGNITIVELY DEMANDING TASKS

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Keywords : dopamine, biosensor, cognitive control, forebrain

Electrophysiological recordings and in silico simulations have established how fast excitatory and inhibitory currents sculpt network representations of attention and working memory (WM) processes. Behavioral studies, however, have demonstrated that slowly evolving psychological states—e.g., arousal, stress, and motivation—affect the performance of cognitively demanding tasks. To date, the mechanisms underlying how behavioral states change the dynamics of the cortical networks during these tasks remain poorly understood. These slowly evolving states are under the influence of ascending neurotransmitter systems such as dopamine (DA), Acetylcholine (ACh), norepinephrine (NE), serotonin (5-HT), etc. Furthermore, these modulatory molecules do not necessarily have direct and fast excitatory or inhibitory effects on their target cells. Neuromodulation, instead, affects the state of neurons or networks of neurons to alter their response to incoming stimuli.

To study the effects of neuromodulation on cortical activity, prior studies have primarily used pharmacological manipulations while monitoring the activity of cortical neurons during behavior. The time course of effects from these drugs (minutes to hours), however, is presumably much longer than the rate of neuromodulator changes under physiological conditions. This has created a critical gap in our understanding of how neuromodulators affect cortical activity. With the advent of optogenetic tools and fluorescent biosensors (such as dLight), we are now able to investigate neuromodulatory actions during normal behavior without the need for pharmacological manipulations. In this talk, I will demonstrate how the application of these sensors to investigate the role of forebrain DA and ACh in working memory, motivated approach, and reward valuation over distinct timescales during instrumental and Pavlovian behavior.

DECIPHERING THE ROLE OF DOPAMINE IN THE MOUSE MEDIAL PREFRONTAL CORTEX

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Keywords: Mesocortical dopamine, prefrontal cortex, attention, fiber photometry

Midbrain dopaminergic (DA) projections to the medial prefrontal cortex (mPFC) exert a powerful neuromodulatory influence over the mPFC and evidence has linked dysregulation of this pathway to an array of neuropsychiatric disorders. In accordance, mPFC DA transmission has been implicated in a variety of behaviorally relevant processes including working memory, cognitive flexibility, and stress reactivity; however, relative to other brain regions whose activity is extensively modulated by midbrain DA systems, the role of mPFC DA transmission remains considerably less well understood. Until recently, investigations of mPFC DA have been prohibitively difficult and limited to either slow, direct measures of extracellular DA such as microdialysis or fast but non-selective measures such as fast-scan cyclic voltammetry. Here, we utilized the fluorescent DA sensor dLight, which has high specificity for DA and sub-second temporal resolution, in conjunction with fiber photometry to parse mPFC DA dynamics in freely behaving mice. Contrary to prior work suggesting that mPFC DA release is preferentially evoked by stressful events, we demonstrate robust DA signal to novel (auditory tone), appetitive (sucrose), and aversive (footshock) stimuli. DA signal to novel stimuli diminished across sessions while the magnitude of responses to sucrose and footshock were proportional to lick bout size and shock intensity, respectively. Interestingly, while we observed no cue-evoked DA signal during an operant task in which animals learned to initiate a correct response for sucrose during a prolonged (30sec) cue presentation period, in a similar task wherein the cue indicating the correct operant response was only presented briefly (1sec) following a variable delay, we observed a cue-evoked increase in DA signal that was preceded by a pronounced decrease in signal during the delay period and that this decrease was highly correlated with task performance. Together, these findings suggest that this dampening of mPFC DA activity serves to filter environmental stimuli to allow selective attention towards a specific expected stimulus, which triggers an mPFC DA transient when detected.

HYPOTHALAMIC BILE ACIDS-TGR5 SIGNALING: A NEW PLAYER IN ENERGY BALANCE REGULATION

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Keywords : Hypothalamus, brain-periphery cross-talk, obesity, lipids, bile acids

Bile acids are critical regulator of metabolic responses in peripheral organs, by particularly involving the activation of the membrane bile acid receptor Takeda-G-protein-receptor-5 (TGR5). Data that we have generated support the existence of a hypothalamic bile acids-TGR5 signalling system, which is particularly relevant to contrast diet-induced obesity. Pharmacological stimulation of central TGR5 reduces food intake and body weight, while decreasing adiposity and improving insulin sensitivity in diet-induced obese mice. These metabolic improvements are due to central TGR5 recruitment of the sympathetic nervous system, which ultimately increases energy expenditure, thermogenesis and lipolysis in adipose tissue. Conversely, genetic downregulation of hypothalamic TGR5 favours obesity in lean mice and worsens obesity in already obese animals, implying a protective role for hypothalamic TGR5 against obesity. Investigations have further defined the cellular and molecular mechanisms involved, thus providing further evidence supporting a beneficial role for hypothalamic bile acids TGR5 signalling in obesity and type 2 diabetes. This project was supported by: INSERM, Aquitaine Region, ANR, FFRD, Mexican ConaCyt.

EXPLORING GUT-BRAIN CIRCUITS TO UNDERSTAND HOMEOSTATIC AND REWARD ADAPTATIONS IN EATING DISORDERS

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Keywords : Vagus nerve, Energy Balance, Homeostasis, Reward, Dopamine

Interoception, a physiological property of paramount importance in the regulation of food intake and energy balance, allows the brain to be constantly informed about the homeostatic states of peripheral organs. Unfortunately, the widespread access to palatable food has led to the development of eating disorders which may be exacerbated by a distorted periphery-to-brain communication. Among a plethora of interoceptive mediators, the vagus nerve represents the main fast neuronal relay which ensures the rapid and dynamic bidirectional communication between the periphery and the brain.

Here, we provide an overview of recent findings indicating that the gut-to-brain vagal axis serves as an integrative lever to scale the hedonic and homeostatic adaptations elicited during compulsive food intake (binge eating) and obesity. In particular, our results highlight the permissive action of peripheral endocannabinoids (eCBs) and the key role of the vagus nerve in governing food reward-associated events and eating disorders. Using a wide array of approaches (motivated behaviours, *in vivo* calcium and dopamine imaging, metabolic efficiency, molecular and structural neuronal plasticity), we have gathered compelling evidence for a yet unappreciated physiological mechanism by which variations of peripheral eCBs control the activity of the vagus nerve, thereby in turn gating the additive responses of both homeostatic and reward brain circuits. Moreover, we are also currently exploring whether this interoceptive circuit may be extended to different reward stimuli and how it may control the activity of the reward system.

In conclusion, our results reveal that the gut-to-brain vagal axis represents an interesting and innovative target which may pave the way to new therapeutic strategies for reward-based disorders.

THE PSTN IN FEEDING BEHAVIOR

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Keywords : Hypothalamus, Neuroanatomy, Central amygdala, Insular cortex

The non-homeostatic component of the feeding behavior relies in part on the reward circuit whose role in the overconsumption of palatable foods is well established. More recently, a circuit whose function seems to be opposite to that of the reward circuit has been described. This circuit is made by connections between the insular cortex (IC), the central nucleus of the amygdala (CEA) and the posterior hypothalamic complex formed by the parasubthalamic nucleus (PSTN) and the Calbindin nucleus (CbN). The PSTN responds to the hedonic value of foods, but data obtained by our team and others indicate that it limits the consumption of such foods. In particular, pharmacogenetic inhibition of TAC-1 neurons in the PSTN affects neophobia or promotes food intake in animals that don't feed because of illness induced by an intraperitoneal injection of LPS. The hypothesis of a participation of the PSTN and the adjacent CbN in states of illness is reinforced by results (c-Fos expression) in rats after acute alcohol intoxication (binge intoxication). In particular, the expression of c-Fos in the CbN is positively correlated with blood alcohol levels.
In conclusion, the IC-CEA-PSTN/CbN network is parallel to the hyperdirect and indirect pathways involving the neighboring subthalamic nucleus. From a functional point of view, it seems to exerts cognitive and interoceptive gating of the feeding behavior.

OBESITY INDUCED DISINHIBITION OF THE ORBITOFRONTAL CORTEX LEADS TO IMPAIRMENT IN GOAL- DIRECTED BEHAVIOUR

Seabrook Lauren[1], Naef, Lindsay[1], Baimel Corey[1], Judge Allap K.[1], Kenney Tyra[1], Tayyab Temoor[1], Ellis Madeleine[1], Qiao, Min[1], Floresco Stan B.[2], Borgland Stephanie L.[1,3]

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Keywords : orbitofrontal cortex, obesity, GABA, disinhibition, goal-directed behaviour

The lateral orbitofrontal cortex (lOFC) receives sensory information about food and integrates these signals with expected outcomes to guide future actions, and thus may play a key role in a distributed network of neural circuits that regulate feeding behaviour. Here, we reveal a novel role for the lOFC in the cognitive control of behaviour in obesity. We show that goal-directed behaviour in obese mice is disrupted such that actions are no longer influenced by the perceived value of the outcome in three reward devaluation tasks; sensory specific satiety, conditioned taste avoidance and contingency degradation. Obesity leads to a reduction in GABAergic input onto and disinhibition of pyramidal output neurons. DREADD-inhibition of local GABAergic input to the OFC impairs sensory specific satiety reward devaluation. Conversely, pharmacological or optogenetic restoration of inhibitory neurotransmission in the lOFC of obese mice reinstates flexible, goal directed behaviour. Our results indicate that obesity hinders an individual's ability to make value representations about rewards, which in turn may influence how individuals make decisions in an obesogenic environment.

AGE-DEPENDENT EFFECTS OF PROTEIN RESTRICTION ON DOPAMINE RELEASE

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Keywords : dopamine, protein, adolescence, fast-scan cyclic voltammetry, diet

Protein restriction at different stages of the lifespan has important long-term consequences on health and development. However, its neurobiological impact remains poorly understood. We previously showed that the dopamine system is highly sensitive to dietary habits during early life periods such as adolescence due to its late maturation. Here, we investigated how a low protein diet (5% versus 18% for control diet) either during adolescence or adulthood in rats impacts dopamine release in the nucleus accumbens (mesolimbic pathway) or in the dorsal striatum (nigrostriatal pathway) using fast-scan cyclic voltammetry on brain slices.

Protein restriction during adolescence, but not adulthood, affects body weight by slowing down animals' growth, despite an increase in their energy intake. Protein restriction increased evoked dopamine release in the nucleus accumbens in adults, but decreased dopamine release in the same brain region in adolescents. Similar results are observed in response to low or high frequency stimulations. In contrast, protein restriction has no effect on dopamine release in the dorsal striatum of adult rats, whereas it induced a frequency-dependent increase of dopamine release in adolescents. These results highlight the complex effects of protein deficiency on different parts of the dopamine system and their vulnerability to unbalanced dietary habits during early life.

DEEP CHEMICAL ANALYSIS OF MICRODIALYSATE BY LC-MS: IN VIVO METABOLOMICS

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Keywords : Microdialysis, Metabolomics, HR/LR model

Brain extracellular space contains a wide range of molecules including neurotransmitters, neuromodulators, and metabolites. The chemical milieu in this space is indicative of cellular activity and is involved in regulation of that activity. Most work to date using microdialysis has focussed on measuring a small number of neurotransmitters or metabolites at a time. Recent advances in LC-MS technology for identifying and quantifying chemicals in complex mixtures have opened the possibility of tracking the concentrations of many more compounds. These trends are exemplified by metabolomics, the field of analysing the full complement of metabolites present in an organism or biofluid. In this work, we describe our efforts to improve and apply the tools of metabolomics to monitoring the brain extracellular space. The goal is to both identify the chemicals present and begin to understand their relationship to phenotype, behaviour, drug effects, or disease state. We also illustrate how these tools can highlight specific pools of glutamate within the brain.

Metabolomics can be performed as a directed or undirected analysis. In directed analysis, a select group of known compounds is determined. In undirected analysis, as many compounds as possible are detected using a general technique like LC-MS. Compounds of interest can be identified by matching the mass spectra to a database. Often only a small fraction of the signals detected can be attributed to specific compound. For example, it is not uncommon to detect 10^4 “features” (signal at a given retention time and mass) but only identify a few hundred compounds.

In this work, we illustrate how directed metabolomics methods can be used to uncover chemical differences of phenotypes, in this case the HR/LR behavioural model. We also describe strategies to identify over 300 compounds in dialysate and illustrate preliminary results using this method uncover unexpected chemical differences in phenotypes. Finally, we describe a LC-MS based method to using stable-isotope tracing to track specific pools of glutamate as a neurotransmitter.

HIGH-RESOLUTION IMAGING OF NEUROMODULATORS USING GENETICALLY ENCODED FLUORESCENT SENSORS

Loïc Duffet[1], Seher Kosar[2], Mariangela Panniello[3], Bianca Viberti[4,5], Edward Bracey[2], Anna D. Zych[6,7], Arthur Radoux-Mergault[8], Xuehan Zhou[1], Jan Dernic[1], Luca Ravotto [1], Yuan-Chen Tsai[1], Marta Figueiredo[1], Shiva K. Tyagarajan[1,3], Bruno Weber[1,3], Miriam Stoeber[8], Nadine Gogolla[6], Markus H. Schmidt[4,5], Antoine R. Adamantidis[4,5], Tommaso Fellin[3], Denis Burdakov[2,9], and Tommaso Patriarchi [1,2,9]

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Keywords : neuromodulators, neuropeptides, genetically-encoded sensors, fluorescent proteins, in vivo imaging

In this talk I will present ongoing work from my lab which aims at developing a next-generation optical toolkit for high-resolution imaging of neuromodulators in living organisms. I will describe the recent development of a new sensor for orexin neuropeptides, which are hypothalamic neuropeptides that carry out essential functions in the central nervous system. Little is known about their release and range of action in vivo owing to the limited resolution of current detection technologies. Our sensor, called OxLight1, is based on the engineering of circularly permutated green fluorescent protein into the human type-2 orexin receptor. In mice OxLight1 detects optogenetically evoked release of endogenous orexins in vivo with high sensitivity. Photometry recordings of OxLight1 in mice show rapid orexin release associated with spontaneous running behavior, acute stress and sleep-to-wake transitions in different brain areas. Moreover, two-photon imaging of OxLight1 reveals orexin release in layer 2/3 of the mouse somatosensory cortex during emergence from anesthesia. Thus, OxLight1 is a novel ultrasensitive probe that enables direct optical detection of endogenous orexin neuropeptides with high spatiotemporal resolution in living animals.

FUNCTIONAL AND MOLECULAR MRI OF DOPAMINERGIC CIRCUITRY IN THE RODENT BRAIN

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Keywords: molecular imaging, fMRI, dopamine, reward, striatum

Although the molecular and cellular effects of dopamine signaling have been extensively studied, their impact on larger-scale neural activity phenomena are less understood. Here we describe functional and molecular imaging studies that place dopaminergic signaling into a broader context of brain-wide neural activity in rodents. First, we describe how molecular-level functional magnetic resonance imaging (fMRI) can be combined with conventional fMRI to elucidate local and global consequences of reward-evoked dopamine release in rat striatum. Using a dopamine-sensitive contrast agent, we measure spatiotemporal profiles of striatal dopamine and study their relationship to hemodynamic activity across the brain. Our results show that dopamine extends the duration of striatal responses to rewarding stimulation and potentiates activity in distal cortical regions associated with motivated behavior. Second, we introduce a genetically encodable imaging probe called NOSTIC that permits selective functional imaging of targeted cell types and circuit elements. By applying the NOSTIC probe using a retrogradely transported viral vector in rats, we identify convergent inputs from dopaminergic and other areas that together shape striatal activity during stimulation. We show that circuit-specific fMRI using NOSTIC provides information distinct from other functional and anatomical measures and propose that this approach may offer a generalizable means for dissection of neural circuit function at a brain-wide level in animals. Finally, we briefly describe novel molecular probes for sensing neurotransmitters and neuromodulators using hemodynamic functional imaging in the mammalian brain. These vasoactive probes act at nanomolar concentrations and may be translatable due to their high potency and potential compatibility with noninvasive brain delivery approaches.

STRESS- AND ETHANOL-INDUCED CHANGES IN STRIATAL EXTRACELLULAR LEVELS OF DYNORPHIN AS MEASURED BY MICRODIALYSIS IN MICE

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Keywords: Dynorphin, Microdialysis, Ethanol, Kappa Opioid Receptor, Stress

Enhanced activity of the neuromodulatory peptide, dynorphin (Dyn), and its target, the kappa opioid receptor (KOR), have been documented during withdrawal from abused drugs and alcohol. This hyperactivity leads to anxiety and dysphoria, which can promote relapse to substance abuse; it is therefore important to understand how the Dyn/KOR system is regulated. We employed microdialysis techniques to collect dynorphin samples from the dorsal and ventral striatum of freely moving mice that have been undergone a Single Prolonged Stress (SPS) protocol (1 day), Chronic Intermittent Ethanol (CIE) vapor exposure (2 weeks), or both. SPS and CIE have been shown to increase anxiety-like behavior and ethanol drinking independently, and we hypothesized that the combination would further augment these behaviors, as well as increase extracellular levels of Dyn, particularly during withdrawal. Our prior studies showed that SPS and CIE each rapidly increased KOR activity, suggesting altered endogenous Dyn signaling. We used 1 mm microdialysis probes with large-pore membranes to allow diffusion of high molecular weight peptides combined with antibody-based ELISA assays for analysis of DYN. To measure “true” basal levels, we used a 100 nL/min aCSF flow rate to allow equilibration between the interior of the probe and the extracellular fluid, and we collected single samples over a range of 2-10 hours, during either the day or night. We found no differences in Dyn levels across the diurnal cycle, between males and females, or in response to CIE, but SPS transiently elevated Dyn levels for 12-24 hours. Transient acute stress induced by yohimbine injection did not change Dyn levels appreciably. We anticipate that Dyn levels will correlate with SPS-induced increases in ethanol drinking and anxiety-like behavior. This would establish the baseline for examining the effects of candidate therapeutic drugs on Dyn levels and drinking.

NANOSCALE APTAMER-MODIFIED BIOSENSORS MONITOR DOPAMINE AND SEROTONIN EX VIVO

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Keywords: DNA, nanobiosensor, nanopore, optoelectronic sensing, multiplex

Measuring specific chemical interactions at the spatiotemporal resolution that approaches biologically meaningful dimensions and timescales is critical for understanding neuronal communication. While advanced methods to record electrical signaling from neurons are prevalent, tools to monitor neurochemical signaling have been limited. We have tackled this challenge by coupling the inherent selectivity of DNA-based recognition elements termed aptamers, with nanoscale pipettes with openings of ca. 10 nm. Aptamers are systematically designed oligonucleotide receptors that exhibit highly specific and selective recognition of targets. Aptamers that recognize small-molecule neurotransmitters, including serotonin and dopamine, have recently been isolated. Upon reversible target binding, aptamers undergo a rearrangement of the negatively charged backbone, and these dynamic structural changes can be transduced as measurable changes in current through the nanoscale orifice of the sensors. Nanoscale confinement of the sensor surface reduces biofouling for long-term recordings in complex environments, overcoming a critical bottleneck for clinical biosensors. We have demonstrated the capacity to detect physiologically relevant differences in neurotransmitter amounts released by live neurons in complex media with unprecedented sensitivity. We are now expanding our technology to measure chemical flux of dopamine in localized brain regions in situ (e.g., striatum, cortex) upon electrical and chemical stimulation of neurotransmitter release. In parallel, we are coupling electronic sensing with optical sensors (genetically encoded dopamine sensors, dLight1) to expand the detection window and enable simultaneous readout of events at nanoscale and cellular resolutions. Further, we aim to target diverse targets beyond serotonin and dopamine through this generalizable method and to multiplex sensors for multi-chemical recordings.

NEUROCHEMICAL MONITORING OF THE BRAIN - THE NEUROINTENSIVE CARE PERSPECTIVE

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Keywords : brain injured patients ; microdialysis ; brain biomarkers ; clinical trials

This talk will provide an overview about invasive neuromonitoring methods for acutely brain injured patients focusing on cerebral microdialysis and brain tissue oxygen tension. Besides technical information and limitations of current available monitoring tools, the clinical value will be discussed based on updated guideline and consensus recommendations. Cerebral microdialysis provides additional possibilities to advanced analytics of brain extracellular fluids. In this context, the value of brain tissue derived biomarkers currently investigated will be discussed: markers of neuroinflammation, neurodegeneration and multi-omics will be weighed against current available treatment strategies and provide insights in future perspectives for clinical trials.

MICRODIALYSIS IN THE INJURED BRAIN

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Keywords: Microdialysis, biosensor, clinical monitoring, traumatic brain injury, spreading depolarization

The potential value of microdialysis in the medical care of patients with severe traumatic brain injury has been amply demonstrated. Even so, clinical microdialysis is not widely used, even in patients that undergo other forms of intracranial monitoring involving, for example, sensors for intracranial pressure and oxygen. Recent enhancements in the approach might increase the feasibility, and thereby the wider adoption, of clinical microdialysis. One such enhancement is the shift away from sample collection and offline analysis in favour of continuously operating real-time sensors. These sensors decrease the labour intensiveness of microdialysis and also deliver medically-relevant responses in near real-time. A second enhancement is the incorporation of dexamethasone in the perfusion fluid for the purpose of mitigating the penetration injury associated with probe insertion. The combination of these enhancements gives rise to dexamethasone-enhanced continuous on-line microdialysis for monitoring glucose and potassium ion in the rodent brain after controlled cortical impact and in the human brain after traumatic brain injury (in this work, mainly due to motor vehicle accidents). Real-time monitoring in the rodent model and in TBI patients has produced a set of similar findings, including observations of spreading depolarization, a mechanism of secondary brain injury, and long-lasting declines in dialysate glucose levels. Unfortunately, we must acknowledge that clinical microdialysis at our institutions has been prevented by covid: we have been unable to monitor patients since the beginning of the pandemic. On-going laboratory studies have been aimed at adapting dexamethasone-enhanced continuous on-line microdialysis for monitoring intracranial oxygen. This is potentially significant because current methods for clinical monitoring of brain O_2 do not include mitigation of penetration trauma. (Acknowledgement: this work has been supported by grants from the US National Institutes of Health R01NS102725, R21NS109875, and 1S10RR028478).

MICRODIALYSIS MONITORING OF CEREBRAL METABOLISM --IN TBI

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Keywords: Microdialysis; ^{13}C-labelling; NMR; mid-IR, TBI (human)

Developments are presented, in three key areas.
[1] Abnormal cerebral metabolism in the acute phase after TBI, during neurocritical care, is statistically associated with patients' clinical outcomes 6 months later, in retrospective data analysis. Variations in cerebral perfusion, oxygenation and glucose supply are associated with changes in cerebral lactate/pyruvate ratio (LPR) measured on a conventional enzymatic-colorimetric bedside microdialysis analyser. These findings suggest therapeutic interventions to improve cerebral metabolism. Future prospective studies are merited to determine the efficacy of these strategies.
[2a] ^{13}C-labelled microdialysis studies have provided clear evidence that lactate can be oxidatively metabolised via the tricarboxylic acid (TCA) by the brain, both TBI and "normal" non-TBI, shown by NMR analysis.
[2b] By targeting LPR using a tiered clinical algorithm incorporating intracranial pressure, brain tissue oxygenation and microdialysis parameters, mitochondrial dysfunction was identified in 73% of TBI patients studied. In these, focal ^{13}C-labelled succinate (disodium salt) administration improved energy metabolism, evidenced by reduction in LPR. NMR analysis of metabolites showed that ^{13}C-succinate was metabolised via the TCA cycle. Succinate merits further investigation for TBI therapy.
[3] Feasibility of using mid-IR spectroscopy has been demonstrated for monitoring the dynamic changes in TBI patients' brain chemistry over several hours and days. Using mid-IR, glucose, lactate, and pyruvate limits of detection were 0.5, 0.2, and 0.1 mM respectively. Further work focuses on improving the sensitivity of mid-IR detection, particularly for pyruvate, as well as demonstrating clinical measurements of continuous online microdialysis monitoring in TBI patients. This will allow sensing of rapid changes in brain chemistry, giving opportunities for timely intervention.
Refs: [1] Guilfoyle et al. 2021 PLoS ONE 16(12):e0260291. [2a] Jalloh et al. 2018 J. Neurotrauma 35:2025–2035. [2b] Khellaf et al. 2022 J Cereb Blood Flow Metab 42(1):39–55. [3] Alimagham et al. 2021 Anal. Chem. 93:11929−11936.

NEW TECHNOLOGIES FOR REAL-TIME PATIENT MONITORING USING MICRODIALYSIS

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Keywords : Microdialysis, biosensor, microfluidic, patient, traumatic brain injury

Injuries to the human brain such as traumatic brain injury can have devastating consequences for the patient that develop over the many days they are in the intensive care unit. Key to this deterioration is secondary brain injury which is a complex multifactorial process. An important component of secondary injury are spreading depolarisation (SDs), profound waves of almost complete neuronal depolarisation that move round the sites of primary injury. We have shown that SDs can be detected by electrophysiology, but their effects on the brain are best demonstrated by continuous neurochemical monitoring, ideally enhanced by the use of local dexamethsome (see Michael talk).

We have been developing a range of novel microfluidic-based technologies to detect neurochemical concentration changes with the high temporal resolution needed to monitor the effects of SDs. In this presentation I will describe a new microfabricated flexible brain sampling probe that can combine areas of membrane for sampling with an array of electrical contacts for electrcorticogaphy. This can then be complemented by a flow through sensor capable of 'imaging' the concentrations of multiple ions in the dialysate stream. The system can feed into a low volume microfluidic device containing biosensors for key energy metabolites. This system is wireless, communicating by Bluetooth to a tablet computer for data visualisation. Finally, we have been developing an on-line data analysis system 'Neuromonitor' to detect key events such as SDs in real-time to increase the clinical utility of the system.

A NOVEL WIRELESS ELECTROCHEMICAL DEVICE FOR REAL-TIME MONITORING OF NEURAL IONIC CHANGES IN PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY

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Keywords: Traumatic Brain Injury, Spreading Depolarisation, Microdialysis, CMOS Technology, Ionophore.

Worldwide, traumatic brain injury (TBI) affects almost 69 million people per year, of which 10-15% of cases are severe, requiring careful clinical care in an intensive therapy unit (ITU) [1]. TBI is an evolving injury that can worsen over time and result into an exacerbation of the neurologic damage, known as secondary brain injury (SBI), which increases the risk of poor outcomes. A continuous and real time monitoring can reveal the underlying mechanisms of the injury and offer medical support to find the best neuroprotective strategy for preventing patients' deterioration.

We present a wireless bedside monitoring device for the detection of ionic changes in cerebral microdialysate streams to identify spreading depolarisation (SD) events, which are disruptive cortical depolarisation waves considered a hallmark of SBIs [2]. We employ an electrochemical device based on Complementary Metal-Oxide-Semiconductor (CMOS) technology that consists of a 3X2mm array of 4756 Ion Sensitive Field Effect Transistors (ISFETs). The sensing surface is coated with ionophore membranes specific to potassium, sodium, and calcium, whose concentration is substantially changed following a SD event [3]. A 3D printed microfluidic flow-cell allows the manipulation of small volumes in the order of uL, which makes the device suitable for the analysis of dialysate streams that are very limited in volume. The flow cell communicates with a programmable microfluidic board, that enables an automated calibration of the device prior the measurement [4]. The recordings are continuously processed and shown in a user friendly and dedicated app. According to preliminary results, the device exhibits a good multi-ion sensitivity with a decent time response which makes its future implementation in ITU settings for a fast detection of SDs very promising.

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[2] Maas et al., The Lancet Neurology 16.12 (2017): 987–1048.
[3] Moser et al., Analytical. Chemistry 92.7 (2020): 5276-528.
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REAL TIME MEASURMENT OF ADENOSINE IN THE VENTROLETERAL PROPTIC NUCLEUS (VLPO): A CRUCIAL HYPNOGENIC MOLECULE

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Keywords: Adenosine biosensors, A_{2A} adenosine receptors, glucose, neuroglial interaction, mouse brain slices.

Adenosine is a purine nucleoside that play a crucial role as an endogenous sleep factor. Systemic or intracerebral injections of adenosine or of its agonist were shown to favor sleep. On the opposite, antagonists of adenosine such as caffeine have a strong wake promoting effect. However, how adenosine levels are locally regulated within the ventrolateral preoptic nucleus (VLPO), the main brain region regulating slow-wave sleep, still remains unexplored. Although it is well established that extracellular adenosine levels allow the brain to assess the need for sleep, its interplay with brain energy metabolism, as well as with other hypnogenic factors such as prostaglandin D2 (PGD2) are crucial elements to better understand sleep physiology.

Here, we aimed to explore the cellular and molecular signaling pathways of adenosine in the VLPO, in relation to circadian rhythm, metabolism and in response to prostaglandin D2. Therefore, we combined real-time detection by *in situ* purine biosensors, infrared videomicroscopy of vascular reactivity, patch-clamp recordings, calcium imaging and immmunolabeling on mouse brain slices. We compared adenosine levels at two different time points of the circadian cycle and quantified adenosine release in response to glucose or PGD2 application. Patch-clamp recordings also uncovered a differential depolarizing effect of adenosine and of its A2A receptor agonist, CGS-21680 on sleep-promoting neurons according to the circadian time.

Altogether, our results provide new insights into the circadian and metabolically driven release of adenosine. These results suggest that glucose and PGD2 might promote sleep, via an astrocytes-derived adenosine and A_{2A} receptors activation, and provide a better understanding of the central role of adenosine in sleep regulation.

IMAGING TOOLS FOR EXTRACELLULAR POTASSIUM AND LACTATE IN BRAIN TISSUE

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Keywords : extracellular lactate; potassium; biosensors; nanosensors; fluorescence

Astrocytes play central roles in the brain for adjusting the levels of several ions, neurotransmitters, and metabolites in order to enable and modulate neuronal transmission. Monitoring these species in the extracellular space with spatial and temporal resolution using imaging approaches is crucial but challenging. Here, I will discuss options for imaging (1) extracellular potassium using the strategy of encapsulating a fluorescent potassium indicator into a dendrimer. The nanosensor, called APG4 PAMAM PEG, has high affinity for potassium enabling optimal detection of this cation in the extracellular space. Characterization and demonstration of its use in situ will be presented; (2) Lactate is accepted as a useful metabolic substrate for brain cells and more recently described to have a signalling role. To image extracellular lactate, we evaluated the suitability of the plasma membrane lactate receptor, hydroxycarboxylic acid receptor 1 (HCAR1, formerly named GPR81), as the basis for the development of a genetically encoded fluorescent lactate biosensor. The potential and current limitations of using HCAR1 as a building block for a fluorescent lactate biosensor will be discussed.

QUANTITATIVE PHASE DIGITAL HOLOGRAPHIC MICROSCOPY TO EXPLORE ASTROCYTE FUNCTIONAL PHENOTYPES

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Keywords : Astrocytes, cell volume, glutamate uptake, functional phenotyping

Quantitative Phase Imaging (QPM) has recently emerged as a powerful new imaging modality to non-invasively visualize transparent specimens, including living cell culture. Among these different QPI techniques, Quantitative Phase Digital Holography Microscopy (QP-DHM) is particularly well suited to explore, with a nanometric axial sensitivity, cell structure and dynamics. Concretely, accurate interferometric measurements of the phase retardation of a light wave when transmitted through living cells are performed. This phase retardation, namely the Quantitative Phase Signal (QPS) depends on both the thickness of the observed cells as well as the difference between its refractive index and that of the surrounding medium. The refractive index difference is generated by the presence of organic molecules, including proteins, DNA, organelles, nuclei present in cells. QPS provides thus information about both cell morphology and cell contents. Thanks to the development of different experimental procedures, this dual information about morphology and intracellular content can be obtained separately allowing the measurement of a whole range of biophysical parameters including cell shape, absolute volume, intracellular protein concentration, nanoscale membrane fluctuations, membrane mechanical properties as well as water permeability and transmembrane water movements.

On the other hand, cell membranes of mammalian are highly permeable to water and movements of water across membranes are therefore dictated in large part by osmotic pressure gradients. Thus, even at constant extracellular osmolarity, cell volume, morphology and the concentration of intracellular compounds of any mammalian cell are permanently challenged by its normal activity, including transport of osmotically active substances, cell metabolism, enzyme activity, which all induce transmembrane water movements for osmotic reasons

STUDY OF THE ASTROGLIAL CONTROL OF THE ANTIDEPRESSANT RESPONSE WITH PHARMACOLOGICAL AND CHEMOGENETIC APPROACHES

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Keywords: antidepressant; astrocytes; gliotransmission, L-AAA, adenosine, DREADD.

Even if major depression is now the most common of psychiatric disorders, successful antidepressant treatments are still difficult to achieve. Therefore, a better understanding of the mechanisms of action of current antidepressant treatments is needed to ultimately identify new targets and enhance beneficial effects.
Given the intimate relationships between astrocytes and neurons at synapses and the ability of astrocytes to "sense" neuronal communication and release gliotransmitters, an attractive hypothesis is emerging stating that the effects of antidepressants on brain function could be, at least in part, modulated by direct influences of astrocytes on neuronal networks.
We will present two studies revealing a permissive role of glia in the antidepressant response: i) Control of the antidepressant-like effects of rat infralimbic-prefrontal cortex (IL-PFC) Deep Brain Stimulation (DBS) by astroglia: it was shown that DBS induced an antidepressant-like response that was prevented by IL-PFC astroglial lesion and by adenosine A1 receptor antagonists, ii) Modulation of antidepressant efficacy of Bright Light Stimulation (BLS) by lateral habenula astroglia; it was shown that chemogenetic activation of lateral habenula (LHb) astroglia prevented the potentiating effect of BLS on the AD response.
In sum, it is proposed that an unaltered neuronal-glial system constitutes a major prerequisite to optimize antidepressant efficacy of DBS or BLS. Collectively, these data pave also the way to the development of safer and more effective antidepressant strategies.

NEURON-DERIVED EXTRACELLULAR VESICLES CONTAINING TAU DISRUPT ASTROCYTES FUNCTION AND MEMORY PERFORMANCE

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Keywords : Astrocytes, Extracellular Vesicles, Tauopathies, Alzheimer's Disease, Mitochondria

Tauopathies are characterized by the accumulation of tau isoforms in different cell subpopulations, including astrocytes. However, the origins and consequences of the different tau accumulations in astrocytes remain unclear. Using microfluidic technology, we demonstrated that neurons accumulating wild-type forms of 3R and 4R tau transfer it to astrocytes. We show that Neuronal Derived extracellular vesicles (ND-EV) play a significant role in propagating 3R and 4R forms to astrocytes. Treatment with tau-accumulating ND-EVs disturbs the astrocytic mitochondrial system (morphology, dynamic and redox state). Injection of tau-accumulating ND-EVs into the hippocampus induced memory impairment in rats. Although no transfer differences between tau isoforms were observed, the effects are significantly more damaging for astrocytes and spatial memory if the EVs originate from neurons accumulating 3R tau than 4R tau. Together, these results indicate that both Tau isoforms transfer from neurons to astrocytes and could affect astrocytic and cognitive function. These results highlight new mechanisms that explain how neurons proteins can spread disease to surrounding astrocytes and alter their functioning.

GUT-BRAIN SIGNALING AND THE CONTROL OF FOOD INTAKE

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Keywords : gut-brain, hunger, food intake, AgRP, calcium signaling

Hunger and our feeding behaviors are tightly regulated by complex and coordinated gut-brain interactions. While we know some mechanisms through which the gut communicates with the brain, our understanding of how nutrients impact *in vivo* neural activity is in its infancy. In our recent work, we discovered the ability of nutrients in the gut to rapidly modulate neural activity in a small population of hunger-sensitive, hypothalamic neurons expressing agouti-related protein (AgRP). Fat, sugar, or amino acids alone are each capable of inhibiting AgRP neuron activity. How are these nutrients in the gut signaled to the brain to update nutritional status in real time? Because individual macronutrients engage specific receptors in the gut to communicate with the brain, we reasoned that macronutrients may utilize different pathways to reduce activity in AgRP neurons. Indeed, we find site-specific differences in intestinal detection of distinct macronutrients by AgRP neurons. We explore the relative roles of vagal, hepatic portal, and spinal afferent signaling in the regulation of AgRP neuron activity and food intake, and demonstrate that different gut-brain pathways can mediate effects of fat vs. sugar on hypothalamic neuron activity. Further, the inhibition of AgRP neuron activity by the post-ingestive effects of macronutrients is almost perfectly correlated with food intake reductions, suggesting that AgRP neuron activity is a strikingly accurate predictor of feeding behavior. Since AgRP neurons drive food intake, engaging these endogenous inhibitory regulators of hunger circuits may inform new and effective weight loss strategies.

ROLE OF ANTERIOR INSULA CIRCUITS IN EMOTIONAL VALENCE AND ANXIETY

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Keywords : emotional valence, anxiety, insula-amygdala circuit, fiber photometry, circuit mapping

(max. 2200 characters including spaces): The response of the insular cortex (IC) and amygdala to stimuli of positive and negative valence were found to be altered in patients with anxiety disorders. However, the coding properties of neurons controlling anxiety and valence remain unknown. Combining photometry recordings and chemogenetics in mice, we uncover the anxiogenic control of projection neurons in the anterior IC (aIC), independently of their projection target. Using viral tracing and ex vivo electrophysiology, we characterize the monosynaptic aIC to basolateral amygdala (BLA) connection, and employed projection-specific optogenetics, to reveal anxiogenic properties of aIC-BLA neurons in anxiety-related behaviors. Finally, using photometry recordings, we identified that aIC-BLA neurons are active in anxiogenic spaces, and in response to aversive stimuli. Together, these findings show that negative valence, as well as anxiety-related information and behaviors, are encoded by aIC-BLA glutamatergic neurons, providing a starting point to understand how alterations of this pathway contribute to psychiatric disorders.

A UNIDIRECTIONAL BUT NOT UNIFORM STRIATAL LANDSCAPE OF DOPAMINE SIGNALING FOR MOTIVATIONAL STIMULI

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Keywords: dopamine release, striatum, basal ganglia, motivated behavior, voltammetry

Dopamine signals in the striatum are critical for motivated behavior. However, their regional specificity and precise information content are under active debate. Dopaminergic projections to the striatum are topographically organized. Thus, we quantified dopamine release in response to motivational stimuli and associated predictive cues in six principal striatal regions of unrestrained, behaving rats. Absolute signal size and its modulation by stimulus value and by subjective state of the animal was inter-regionally heterogeneous on a medial to lateral gradient. In contrast, dopamine-concentration direction of change was homogeneous across all regions: appetitive stimuli increased and aversive stimuli decreased dopamine concentration. Although cues predictive of such motivational stimuli acquired the same influence over dopamine homogeneously across all regions, dopamine-mediated prediction-error signals were restricted to the ventromedial, limbic striatum. Together, our findings demonstrate a nuanced striatal landscape of unidirectional, but not uniform, dopamine signals, topographically encoding distinct aspects of motivational stimuli and their prediction.

DIFFERENTIAL ROLE OF ACCUMBAL OPIOID PEPTIDES IN FOOD REWARD AND STRESS

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Enkephalin, Dynorphin, Nucleus accumbens shell, stress, food reward

Opioid peptides are key modulators of reward and stress processing. Recent evidence from our lab and others shows that opioid peptide dynamics and function within the nucleus accumbens shell (NAcSh) yields differential and often opposing motivated behaviour states. Here, we show that enkephalinergic neurons in the ventral NAc shell (vNAcSh) are activated following exposure to predator odor and experimenter handling using fiber photometry. To get a deeper insight into enkephalin release dynamics we couple microdialysis and nano-liquid chromatography/mass spectrometry to allow the detection of endogenous Leu- and Met-enkephalin *in vivo*. We show that enkephalins are released following exposure to predator or handling stress as well as investigating the dynamics of Met-and Leu-enkephalin release and how they correlate to one another in the ventral NAc shell. In contrast we identify a unique role for dynorphin in feeding behavior that is restricted to the dorsal NAcSh (dNAcSh). Photostimulation of dNAc dynorphin neurons robustly attenuates feeding behavior and blunts reward-seeking and consumption in a sex-specific manner. Following acute food restriction and deprivation, photostimulation of dNAc dynorphin neurons continues to block reward seeking and consumption. Together, these data highlight the unique role of accumbal opioid peptides in food reward and stress.

SUB-SECOND FLUCTUATIONS OF EXTRACELLULAR DOPAMINE IN HUMANS ENCODE *VALENCE -PARTITIONED* REWARD AND PUNISHMENT PREDICTION ERRORS

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Keywords: human voltammetry, dopamine, prediction error, reinforcement learning, subjective experience

The rate at which action potentials are generated at the soma of dopamine releasing neurons has been shown to encode temporal difference (TD) reward prediction errors (RPEs). Downstream, the release of dopamine into the extracellular space is not guaranteed to reflect this calculation. Data from non-human and human experiments suggest that fluctuations in extracellular dopamine levels on sub-second timescales is more complex than the long-standing TD-RPE hypothesis about dopamine neuron activity – including data that support the idea that dopamine release may occur to aversive or salient stimuli. Here, we test the hypothesis that dopamine release in human striatum encodes TD-RPE using fast scan cyclic voltammetry performed during brain surgery. Patients with essential tremor undergo brain surgery to implant deep brain stimulating electrodes to manage chronic debilitating motor symptoms. These patients are awake during the procedure and, by all accounts, have an intact dopaminergic system. This permits an opportunity to measure and investigate the role of sub-second changes in extracellular dopamine levels in awake humans while they perform experimental tasks. We demonstrate that dopamine release does encode TD-RPEs when outcomes are rewarding (or expected to be rewarding), but not when outcomes are (or expected to be) punishing. We propose a *valence-partitioned* model for efficient learning of appetitive and aversive events and show that this model is a better explanation of dopamine release in these experiments. Notably, our new approach retains optimal learning rules from TD-reinforcement learning theory but extends the algorithm to allow independent encoding and learning of aversive experience. This approach explicitly accounts for the possibility that different sub-populations of (dopamine or other neuromodulatory) neurons may encode rewarding versus punishing events. Further, this model allows for a parsimonious explanation regarding the relationship between prediction error signaling at the soma and aversive or saliency encoding at release sites throughout the mammalian brain.

NEW FLUORESCENT BIOSENSORS FOR INTERSTITIAL CALCIUM

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Keywords : Bio-Imaging, Calcium, Fluorescent Proteins

Based on our previous work on FRET-based “Twitch” sensors we generated a family of single fluorophore sensors that incorporate a small domain of Troponin C into the fluorescent protein mNeonGreen. These sensors were optimized intensively using directed evolution and screening approaches, generating a number of variants with large fluorescence changes, different Kds and kinetic properties. Compared to the widely used GCaMPs, GreenTs bind fewer calcium ions per sensor (1-2 versus 4), are smaller in size, promise better biocompatibility and retain the N-and C-termini of mNeonGreen for adding targeting sequences. We generated low affinity variants (GreenT-ECs) of these sensors suitable for monitoring calcium in interstitial fluids and spaces and targeted them to the cell surface. When expressed in brain slices or transgenic zebrafish the sensors allow imaging calcium transients in response to various types of intervention. Thus, GreenT-ECs show particular promise for studying calcium regulation in tissue interstitial spaces.

BRET BIOSENSORS FOR LIVE IMAGING OF ERK AND MTOR SIGNALING PATHWAYS ACTIVATION IN NEURONAL PLASTICITY IN VIVO

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Keywords : Biosensor, ERK/mTOR signaling, BRET microscopy, Fiberscope, in vivo brain imaging.

Bioluminescence Resonance Energy Transfer (BRET) imaging allows monitoring of protein-protein interaction in living cells. We engineered intramolecular BRET-based biosensors, named YEN and AIMTOR to report ERK and mTOR activities, respectively [1], [2]. Subcellular compartment-specific versions were also designed. mTOR and ERK are essential kinases involved in the regulation of various physiological processes ranging from cell proliferation and differentiation to cell survival and metabolism. Their kinase activities are spatiotemporally regulated in a signal-specific manner. We characterized YEN and AIMTOR biosensors using single-cell BRET microscopy to investigate ERK/mTOR signaling dynamics in living cells. In hippocampal neuronal cultures, we observed changes in BRET intensities following transient enhanced neuronal activity indicating ERK and mTOR pathways activation. YEN and AIMTOR further revealed ERK/mTOR-signaling dysfunctions in neurons from mouse models of autism spectrum disorders. Built on previous developments [3, 4], we constructed a new fiberscope that combines BRET and fast red fluorescence imaging with optical sectioning, allowing photostimulation of neurons with a cellular level spatial resolution. This fiberscope can acquire bioluminescent signals in the spectral range of BRET sensors, thanks to an optimized luciferase substrate. Using YEN and AIMTOR BRET-based sensors, our goal is now to assess ERK or mTOR signaling pathways activation correlated to Ca2+ transients using the red fluorescent jRGeCo biosensor in vivo in freely behaving mice.

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BIOSENSOR IMAGING TO REVEAL THE SPECIFICITIES OF CAMP/PKA SIGNAL INTEGRATION IN THE STRIATUM

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Keywords: Biosensor imaging; cAMP/PKA signalling; dopamine; striatum

Striatal Medium-sized Spiny Neurones (MSNs) integrate dopamine signals through the cAMP-PKA signalling pathway, but how this process takes place in the context of several neuromodulators being present in a timely manner in the brain remained hard to study. Genetically encoded biosensors are wonderful tools to image in real time the dynamics of the cAMP/PKA response to dopamine in mouse brain slices. At the level of individual neurones, this approach clearly confirmed the segregation of dopamine D1 and D2 receptors in two totally separate sub-classes of MSN. This dynamic approach also reported the net result of antagonistic neuromodulatory actions: Gi/o-coupled receptors, such as dopamine D2 and muscarinic M4 efficiently suppressed the positive cAMP response triggered by Golf-coupled receptors such as dopamine D1 and adenosine A2A. This is consistent with a gating action of acetylcholine, which must be down for dopamine to increase cAMP through D1 receptors. Phosphodiesterases (PDE) are crucial regulators of this cAMP dynamics, and a number of specific and brain-penetrant inhibitors have been developed recently to treat various neurological and psychiatric diseases. With biosensor imaging, PDE10A emerged as the centrepiece of dopamine signalling in MSNs, while PDE1, PDE2A and PDE4 played a more subtle role in the fine-tuning of the response. Finally, we observed that MSN responded to brief dopamine inputs with a high sensitivity through a non-linear process at the level of PKA-dependent phosphorylation: an all-or-none responsiveness depends on a very specific set of signalling proteins, such as DARPP-32, that amplifies the PKA response through a phosphatase inhibition mechanism. This differs profoundly from what was observed in several other brain regions, where cAMP/PKA responses fade away as the signal progresses from the membrane through the cytosol and into the nucleus. Biosensor imaging thus uncovered the peculiarities of striatal neurones, which appear tailored to detect brief dopamine signals Such basic understanding of signal integration dynamics in situ is critical to envision novel therapeutic approaches which, for example, target striatal phosphodiesterases.

CONTROL OF CORTICAL AXON MORPHOGENESIS THROUGH MITOCHONDRIA TRAFFICKING AND FUNCTION

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Keywords : Axon ; Mitochondria ; Metabolism ; Kinases ; Neurodevelopment

The proper function of neuronal circuits in the adult brain relies on glucose metabolism to ensure energy-demanding neuronal functions such as synaptic activity or long-distance axonal transport. Deregulation of the energy metabolism is strongly associated to many neurodegenerative diseases and has been linked to some neuropsychiatric diseases such as schizophrenia. However, our current understanding of metabolic regulation in the developing brain and in particular in rapidly growing neurons is still fragmental.

We previously identified a signaling pathways involving two kinases LKB1 and NUAK1, and controlling cortical axons outgrowth and terminal branching through a novel mechanism involving the regulation of mitochondria trafficking and clustering in the developing axon. My presentation will review the latter findings, as well as our use of molecular tools to visualize mitochondria trafficking and function in cultured neuron.

A NEAR INFRARED FLUORESCENT SENSOR PAINT TO IMAGE DOPAMINE SIGNALING

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Keywords : Dopamine, fluorescence microscopy, sensors, exocytosis, signalling

Cells use molecules to exchange information. A very prominent example is neurotransmitter signaling between neuronal cells. The neurotransmitter dopamine is released from discrete axonal structures called varicosities. Its release is essential in behaviour and is critically implicated in prevalent neuropsychiatric diseases but existing dopamine detection methods are not able to detect and distinguish discrete dopamine release events from multiple varicosities. This prevents an understanding of how dopamine release is regulated across populations of discrete varicosities. Using a near infrared fluorescent (980 nm) dopamine nanosensor 'paint' (AndromeDA) based on single-walled carbon nanotubes (SWCNTs), we show that action potential-evoked dopamine release is highly heterogeneous across release sites and also requires molecular priming. Using AndromeDA, we visualize dopamine release at up to 100 dopaminergic varicosities simultaneously within a single imaging field with high temporal resolution (15 images/s). We find that 'hotspots' of dopamine release are highly heterogeneous and are detected at only ~17% of all varicosities. In neurons lacking Munc13 proteins, which prime synaptic vesicles, dopamine release is abolished during electrical stimulation, demonstrating that dopamine release requires vesicle priming. In summary, AndromeDA reveals the spatiotemporal organization of dopamine release.

MICRODIALYSIS AND BEYOND FOR MULTIPLEXED NEUROCHEMICAL MONITORING

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Keywords: Dopamine, serotonin, voltammetry, machine learning, optogenetics

New methods and technologies precede discoveries in biology and medicine. We develop and/or deploy methods and technologies that enable *in vivo* neurotransmitter monitoring. Numerous neurochemicals with diverse structures act in a concerted manner to encode, store, retrieve, and utilize information in brains. Thus, we aim for multiplexed monitoring. Microdialysis sampling coupled with instrumental analysis is inherently multiplexed. We have focused on coupled serotonin and dopamine measurements *via* microdialysis coupled to HPLC-ED with 1-5 min online temporal resolution to investigate the intergenerational effects of maternal stress and antidepressant administration on offspring hippocampal function. In addition to microdialysis, we developed rapid pulse voltammetry (RPV) with machine learning for data analysis for simultaneous serotonin and dopamine monitoring at faster timescales (5-10 Hz). Both microdialysis and RPV revealed coupled striatal dopamine and serotonin release in response to selective optogenetic activation of midbrain dopamine neurons. We have also developed electronic biosensors to investigate chemical signaling. Target recognition is by oligonucleotide receptors (aptamers) linked to field-effect transistor (FET) arrays for transducing binding events. We have selectively detected serotonin, dopamine, glucose, phenylalanine, and cortisol in a label-free manner in physiological fluids over 5□6 orders of magnitude with pM□fM detection limits. We deployed neuroprobes with aptamer-FET sensors on stiff and flexible substrates and monitored serotonin in the brain and spinal cord at physiological concentrations. We are advancing multiplexed electronic biosensors for integrated target monitoring.

Andrews, A. M., The BRAIN Initiative: Toward a chemical connectome. *ACS Chem Neurosci* **2013,** *4*, 645.

ELECTROCHEMICAL APTAMER-BASED BIOSENSORS FOR PHARMACOLOGY AND NEUROSCIENCE

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Keywords : Biosensor, Aptamer, Neuropharmacology, Real-time Monitoring, Behavior

Drugs exert their behavioral effects via concentration-dependent actions upon neural circuits. Yet there has been limited attention to the role pharmacokinetics (PK) plays in psychopharmacology because the temporal resolution with which we can measure drugs is often *orders of magnitude* too slow to capture the concentration changes associated with altered physiological and behavioral processes. For instance, procaine and cocaine are psychoactive compounds that have short half-lives and elicit highly dynamic behavioral effects upon intravenous delivery making determination of the relation between in-brain concentration and on-going behavior challenging. Here, we describe the electrochemical aptamer-based (E-AB) sensor platform that is: (1) receptor-based, rendering it independent of the chemical reactivity of its targets and thus generalizable; (2) able to monitor molecules at physiological relevant time scales in the brains of awake, freely moving animals, and (3) real-time making it capable of supporting closed-loop control of molecular concentrations in brain. Supporting the utility of E-ABs for in-brain molecular monitoring, we report EAB sensors that exhibit sufficient sensitivity, appropriate temporal resolution (~12s), and stable drift characteristics to fully resolve in-brain PK of 3 drugs in individual rats. These data combined with locomotor measurements enable detailed analyses of the relations of in-brain concentration to behavioral response for each subject. Next, we employ EAB-supported feedback-control of drug concentration to remove individual differences in PK. In conclusion, we have developed technology capable of determining individual, in-brain PK of drugs in behaving animals enabling unprecedentedly detailed concentration-response analyses. Moving forward, we are developing EAB sensors against neurotransmitters to allow simultaneous monitoring of drugs and the neurochemical systems that they impact.

A WINDOW TO THE BRAIN: ON-LINE MEASUREMENT OF BIOLOGICS CONCENTRATIONS AND TARGET ENGAGEMENT USING MICRODIALYSIS AND CEREBRAL OPEN-FLOW MICROPERFUSION

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Keywords: Microdialysis; absolute quantification; antibodies; interstitial fluid; open flow microperfusion.

The determination of concentrations of large therapeutic molecules, like monoclonal antibodies (mAbs), in the interstitial brain fluid (ISF) is one of the cornerstones for the translation from preclinical species to humans of treatments for neurodegenerative diseases. Microdialysis (MD) and cerebral open flow microperfusion (cOFM) are the only currently available methods for extracting ISF, and their use and characterization for the collection of large molecules in rodents have barely started. For the first time, we compared both methods at a technical and performance level for measuring ISF concentrations of a non-target-binding mAb, trastuzumab, in awake and freely moving mice. Without correction of the data for recovery, concentrations of samples are over 10-fold higher through cOFM compared to MD. In vivo recovery (zero-flow rate method) revealed an increased extraction of trastuzumab at low flow rates. Technical optimizations have significantly increased the performance of both systems, resulting in the possibility of sampling up to 12 mice simultaneously as well as sampling from the same group of mice over two different time periods. Moreover, strict aseptic conditions have played an important role in improving data quality. The standardization of these complex methods makes the unravelling of ISF concentrations attainable for various diseases and modalities, starting in this study with mAbs, but extending further in the future to RNA therapeutics, antibody-drug conjugates, and even cell therapies. The possibility to perform PK/PD measurements in the same ISF samples is also now achievable and enable to further investigate target engagement in the relevant brain compartment which the interstitial space represents.

Disclosures: Florie Le Prieult is employee of AbbVie and may own AbbVie stock. AbbVie sponsored and funded the study; contributed to the design; participated in the collection, analysis, and interpretation of data, and in writing, reviewing, and approval of the final publication.

A MICROFLUIDIC SAMPLING DEVICE BASED ON TWO-PHOTON POLYMERIZATION FOR INVESTIGATING PROCESSES IN BRAIN

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Keywords : Electroosmosis; Peptides, Peptidases, Capillary LC, Mass Spectrometry

We determined in the mid-2000s that brain tissue has a significant zeta potential. As a result of this tissue property, brain tissue supports electroosmotic flow (EOF). We have used EOF in a push-pull arrangement to determine the rates of hydrolysis of leucine enkephalin in the CA1 and CA3 areas of intact rat organotypic hippocampal slice cultures. This was part of an effort to understand the relative sensitivity of CA1 and CA3 to oxygen/glucose deprivation (OGD). Based on inhibition by somewhat specific peptidase inhibitors and the measured value of the Michaelis constant, we deduced that aminopeptidase N activity differed in the two regions. Further experiments confirmed that this difference in aminopeptidase N activity was responsible for the observed differences in the relative sensitivity of the two regions to OGD.

In order to ask similar questions in vivo, a reproducible means for doing push-pull in the brain must be found. Thus, we created devices using two-photon polymerization (Nanoscribe). A model (a 3D drawing) of a push-pull device is made in SolidWorks or COMSOL. This model is used to create the physical object The device is connected to fused silica capillaries for electroosmotic delivery of substrate peptide (with a D-amino acid internal standard). The first-generation device was attached to a microdialysis (MD) probe for measuring remaining substrate and product concentrations. The second-generation device has the MD probe outside the head. The electroosmotic flow passes by the MD probe, and the MD effluent passes fluid to online LC-MS/MS for quantitative analysis.

WIRELESS, BATTERY-FREE PUSH-PULL MICROSYSTEM FOR MEMBRANE-FREE NEUROCHEMICAL SAMPLING IN FREELY MOVING ANIMALS

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Keywords : neural probes, neurochemical sampling, wireless and battery-free

Extensive studies across multiple animal models and humans have demonstrated that neurochemicals with large molecular weights, such as neuropeptides and other polypeptide neurochemicals, play critical roles in various neurological disorders. Despite many attempts, existing methods such as microdialysis and fast-scan cyclic voltammetry (FSCV) have limited spatiotemporal precision and molecular specificity in detecting these larger molecules, which potentially leaves the molecular mechanisms underlying many neurological and psychological disorders unresolved. Here, we report a wireless, programmable push-pull microsystem for membrane-free neurochemical sampling with cellular spatial resolution in freely moving animals. In vitro studies demonstrate the sampling of various neurochemicals with high recovery (>80%). Open-field tests reveal that the device implantation does not affect the natural behavior of mice. The probe successfully captures the pharmacologically evoked release of neuropeptide Y in freely moving mice. This wireless push-pull microsystem creates opportunities for neuroscientists to understand where, when, and how the release of neuropeptides modulates diverse behavioral outputs of the brain.

INCREASED ATP RELEASE, ATP-DERIVED ADENOSINE FORMATION AND ADENOSINE A_{2A} RECEPTOR DENSITY AS DETERMINANTS OF BRAIN DYSFUNCTION

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Keywords: adenosine, ATP, ecto-nucleotidases, neuropsychiatric diseases

The regular consumption of moderate doses of coffee is associated with increased healthspan (N Engl J Med 2012; 366:1891). One coffee component, caffeine, has been shown to attenuate different neuropsychiatric diseases through the antagonism of adenosine A_{2A} receptors ($A_{2A}R$) (J Neurochem 2016; 139:1019). Adenosine is a stress signal, which is either directly produced in an activity-dependent manner or formed from the extracellular catabolism by ecto-nucleotidases (namely CD73) of ATP released as a danger signal in the brain (Front Neurosci 2015; 9:148).

We now tested if the overactivation of $A_{2A}R$ that triggers neuronal damage is due to increased formation of ATP-derived adenosine and/or increased $A_{2A}R$ density. This implies quantifying ATP release, the density and activity of CD73 and the density/ expression of $A_{2A}R$ in different animal models of brain dysfunction.

Aged rats, APP-PS1 mice and mice icv-challenged with B-amyloid to mimic early Alzheimer's disease (AD), all displayed memory deficits accompanied by increased ATP release from nerve terminals, increased CD73 density, increased $A_{2A}R$ expression and synaptic density in the hippocampus. Pharmacological and optogenetic $A_{2A}R$ overactivation is sufficient to trigger memory impairment in normal rats and mice (Br J Pharmacol 2015; 172:3831; Mol Psychiatry 2015; 20:1339) and the blockade of either $A_{2A}R$ or CD73 prevented memory impairment in aging or AD models. This shows that an overfunction of the whole ATP-CD73-$A_{2A}R$ axis is required to trigger memory dysfunction. This is also observed in the hippocampus of kainate-challenged mice (mimicking temporal lobe of epilepsy) and of repeatedly restrained stressed rats (mimicking depression) and in the striatum of 6-OHDA-treated rats (mimicking Parkinson's disease).

These findings show that brain dysfunction requires a simultaneous increase of $A_{2A}R$ and of ATP-derived extracellular adenosine formation. This prompts considering increased levels of ATP, CD73 and $A_{2A}R$ as a biomarker of brain dysfunction. (Supported by La Caixa Foundation, HP17/00523; Centro 2020, 01-0246-FEDER-000010).

ADENOSINE RECEPTOR HETEROMERS: BIASING ANTIPSYCHOTICS

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Keywords: Antipsychotics; Adenosine receptors; Heteromerization; Dopamine receptors; Schizophrenia.

The dopamine D_2 receptor (D_2R) is the common target of clinically used antipsychotics. The highest D_2R density is found in the striatum, where is it expressed together with the adenosine A_{2A} receptor ($A_{2A}R$) on medium spiny neurons. It is well-established that allosteric interactions take place between these two receptors. However, the extent to which these interactions affect antipsychotic actions is incompletely understood. We recently demonstrated a significant and pronounced reduction of $A_{2A}R$-D_2R heteromers in postmortem caudate nucleus from schizophrenic subjects, even though both D_2R and $A_{2A}R$ were upregulated. Interestingly, similar observations were obtained in the PCP animal model of sensory gating impairment of schizophrenia. Importantly, in this animal model of psychosis the sub-chronic administration of haloperidol or clozapine counteracted the reduction of striatal $A_{2A}R$-D_2R heteromers. Thus, the degree of $A_{2A}R$-D_2R heteromer formation in schizophrenia might constitute a hallmark of the illness, which indeed should be further studied to establish possible correlations with chronic antipsychotic treatments. Accordingly, we examined the effect of typical and atypical antipsychotic drugs on $A_{2A}R/D_2R$ heteromer formation dynamics. Interestingly, computational modelling revealed a distinctive binding mode of clozapine at the D_2R, thus impacting on $A_{2A}R$-D_2R heteromer stability. While clozapine, haloperidol, and aripiprazole all attenuated phencyclidine-induced hyperlocomotion to some extent both in $A_{2A}R^{+/+}$ and $A_{2A}R^{-/-}$ mice, the efficacy of clozapine was most affected by $A_{2A}R$ deletion. Hence, the beneficial effects of clozapine in psychosis might be mediated by a reduction of $A_{2A}R/D_2R$ heterodimers combined with direct D_2R antagonism. Overall, we propose that striatal $A_{2A}R/D_2R$ heteromers may represent an interesting therapeutic target in schizophrenia.

DEVELOPING TOOLS TO MEASURE DYNAMIC GUANOSINE SIGNALING IN THE BRAIN

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Keywords : adenosine, guanosine, fast-scan cyclic voltammetry, microfluidics, ischemia

Guanine-based purines have historically been studied as intracellular modulators of G-proteins; recently, a putative role of extracellular guanosine induced neuromodulation has disrupted the paradigm that guanine-based purines function primarily intracellularly. Recently, guanosine was proposed to be a multi-target neuromodulator, involved in ischemia, pain, mood, seizures and neurodegenerative disorders. Despite this knowledge, the mechanism of guanosine release, clearance, and function in response to neurological injury, like ischemia, remains contradictory in the literature, partially due to the lack of analytical tools to specifically probe guanosine within spatially resolved regions of tissue injury in real-time. To fill this gap, our lab has recently developed a fast voltammetric technique using fast-scan cyclic voltammetry (FSCV) to monitor subsecond transient events of guanosine. Additionally, we have coupled voltammetry with microengineered platforms to aid in studying local fluctuations in guanosine at the site of focal ischemia in the brain. This talk will focus on the development, coupling, and application of these techniques to better understand guanosine's role during normoxia and severe ischemia. Ultimately, this work will set the foundation for measuring local guanosine dynamics at the site of injury with significantly improved spatiotemporal resolution which will provide critical information of the brain's immediate local damage response.

REGULATION OF SPONTANEOUS AND MECHANOSENSITIVE ADENOSINE RELEASE

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Keywords : adenosine, fast-scan cyclic voltammetry, A1 receptor, pannexin, ischemia

Our lab has identified several modes of rapid adenosine release, including spontaneous adenosine release, which is unstimulated, and mechanosensitive adenosine release, which is stimulated by mechanical movement in the brain. Here, we will describe the regulation of both spontaneous and mechanosensitive adenosine release. To understand how adenosine receptors regulate the two modes of adenosine, we used global A1 or A2A knockout mice. A1KO mice have increased frequency of spontaneous adenosine events, but no change in the average concentration of an event, while A2AKO mice had no change in frequency but increased average event concentration. Mechanically-stimulated release was largely unregulated by A1 and A2A receptors, likely because it is released by a different mechanism than spontaneous adenosine. We also tested pannexin 1 channels as a mechanism of release. Deletion of Pannexin 1 channel decreases the concentration of mechanically-stimulated adenosine concentration but does not change spontaneous adenosine release. Finally, we looked at the distance of diffusion for adenosine for spontaneous and mechanically-stimulated release using dual-channel FSCV. Spontaneous adenosine was very local, and transients were not detected 50 um away. Mechanosensitive release diffused further, up to 150 um. Spontaneous adenosine increases during ischemia in some areas, so understanding the mechanism of adenosine regulation are important for understanding its mode of operation as a neuromodulator. These results point to different regulation and mechanisms of release for spontaneous and mechanically-stimulated adenosine, which could be differently regulated for treatments for disease.

IMPACT OF HIPPOCAMPAL UPREGULATION OF ADENOSINE A_{2A} RECEPTORS IN THE MOUSE HIPPOCAMPUS

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Keywords : Adenosine A_{2A} receptor, Astrocyte, Hippocampus, Memory

Alzheimer's disease (AD) is characterized by the intraneuronal aggregation of tau proteins which leads to synaptic dysfunctions and memory decline. Studies have reported that chronic caffeine consumption reduces AD risk and cognitive deficits. These protective effects would be ascribed to the blockade of adenosine A_{2A} receptors (A_{2A}Rs) which are pathologically upregulated in hippocampal astrocytes of AD patients and whose levels have been correlated with the development of cognitive deficits. However, the mechanisms underlying the link between astrocytic A_{2A} upregulation and cognitive deficits remain unknown.
To uncover the effects of astrocytic A_{2A}R upregulation, we intrahippocampally injected an AAV2/9 virus allowing A_{2A}R expression (AAV-A2A), or GFP as control (AAV-GFP), under a GFAabc1d astrocyte-specific promoter, in 2m-old C57Bl6/J mice. We then evaluated impact of astrocytic A_{2A}R upregulation on spatial memory performances, response to hippocampal neural networks using DREADD technology as well as astrocyte reactivity, morphology and transcriptome.

Our data show that A_{2A}R overexpression in hippocampal astrocytes impairs short-term spatial memory and long-term spatial learning. At the network level, thanks to a DREADD approach, we observed an enhanced neuronal activability of hippocampal neurons following A_{2A}R astrocytic overexpression. These neuronal changes were associated with changes in astrocyte reactivity, 3D morphology and transcriptome.

These results demonstrate that A_{2A}R upregulation in hippocampal astrocytes, as seen in the brain of AD patients, is sufficient to alter the astrocytic phenotype, neuronal response and memory. Further experiments in AD mouse models will determine how this A_{2A}R deregulation in astrocytes potentiates the development of AD lesions and their consequences, but also whether astrocytic A_{2A}R downregulation is sufficient to bring benefits.

Our projects are supported by ANR ADORASTrAU, Fondation Alzheimer (ADOMEMOTAU) and LabEx DISTALZ. AL is supported by a doctoral grant of FRM (ECO202106013670).

EXPANDING NEUROCHEMICAL MEASUREMENTS IN ZEBRAFISH

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Keywords : zebrafish, fast-scan cyclic voltammetry, zinc, dopamine, oxytocin

Zebrafish are rapidly emerging as a model of neurological function. Brains from zebrafish offer an ideal compromise between size and complexity. They are small enough to remain viable in a superfusion chamber for hours, yet they contain many neuronal pathways analogous to those found in mammals. This presentation will examine neuronal function in whole, living zebrafish brains. We will present our efforts to measure the light-initiated release of endogenous oxytocin with fast-scan cyclic voltammetry. Toward this end, we have optimized the waveform for oxytocin. We then applied this method to measure sub-second oxytocin release in whole brains harvested from zebrafish that express channel rhodopsin and yellow fluorescent protein on oxytocinergic neurons. Electrode positioning was aided by fluorescence microscopy, and oxytocin was stimulated by the application of blue light. We have not only measured light-evoked oxytocin release, but also spontaneous oxytocin release transients that occur over the course of minutes. We will also present work in which we have used caged zinc compounds to determine how the sub-second application of free, ionic zinc (Zn^{2+}) regulates dopamine release in the zebrafish telencephalon. Zn^{2+} is released from select populations of neurons and can modulate neuronal function by acting on multiple protein molecules, including AMPA receptors and dopamine transporters. We induced the photorelease of Zn^{2+} by applying sub-second pulses of light; thus, our uncaging method is designed to mimic these Zn^{2+} release events. Our findings collectively expand the toolkit for measuring neurochemical signaling in this valuable research model.

LAT1 DEPENDENT AMINO-ACID TRANSPORT AND SLEEP/WAKE REGULATION, INVESTIGATION USING HPLC IN *DROSOPHILA*

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Keywords: drosophila; sleep; dopamine; amino acid transport; nutrients

Sleep and wakefulness have a pervasive impact on neurotransmission, nutrient supply and waste elimination, and thus on the coordination of numerous cellular transport systems. To investigate the mutual interactions between vigilance states and nutrients transport, we used the molecular genetic tools of *Drosophila* to downregulate members of the LAT1 (Large neutral Amino-acid Transporter 1) family of transporters in dopaminergic neurons or in surface glia, the fly equivalent of the blood brain barrier (BBB). LAT1 is responsible for the import of large branched and aromatic neutral amino-acids, and in particular essential amino-acid that are precursors for monoamines synthesis. In the mammalian central nervous system, LAT1 is expressed at high levels in the BBB, and is involved in the import of the amino-acid L-DOPA used in Parkinson's disease treatment.

We show that fly *Jhl-21* and *minidiscs (Mnd)* LAT1-like transporters, are required in dopaminergic neurons and in surface glia for sleep/wake regulation. Down-regulating either gene in dopaminergic neurons resulted in higher sleep amounts during the night, suggesting a defect in dopaminergic transmission. Conversely, downregulating these two genes in surface glia results in reduced sleep during the early part of the night. Since LAT1 can regulate TOR (Target Of Rapamycin) signaling, we investigated the role of this amino-acid sensing pathway and find that it similarly modulates sleep/wake states.

We assessed amino-acid transport efficiency by L-DOPA feeding, which induces insomnia and strongly enhances dopamine synthesis in flies. Downregulation of *Jhl-21* and *TOR*, but not *Mnd*, reduced the sensitivity to L-DOPA as measured by sleep loss, but had no effect on dopamine synthesis, as evaluated using HPLC on single drosophila brains. *Jhl-21* downregulation also attenuated the sleep loss induced by driving constitutively dopaminergic neurons activity. Altogether, this study provides evidence that LAT1 mediated amino-acid transport in dopaminergic neurons and in surface glia is playing a significant role in sleep/wake regulation, providing entry points to elucidate the role of nutrients such as amino-acids in this context.

INTRACELLULAR ELECTROCHEMISTRY IN DROSOPHILA SHOWS EXOCYTOSIS IS PARTIAL AND COMPLEX

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Keywords: Drosophila, amperometry, vesicles, exocytosis, partial release

Recent chemical nanomethods at endocrine model cells have been used to show that the 70-year old concept put forward by Bernard Katz of 'all-or-none' exocytosis of molecules from neurons is suspect. Micro-/nano-electrochemical approaches have been developed to realize the quantitative measurement of intravesicular content and real-time monitoring of their release dynamics. Our group has developed new techniques based on vesicle impact electrochemical cytometry (VIEC) and intracellular VIEC (IVIEC) to provide a highly effective way to quantify the electroactive contents inside vesicles. This has allowed direct comparison of the quantity of molecules released by exocytosis to those in the vesicles. Partial release is observed across all cell types examined to date by the electrochemical methods and appears to be regulated.

We have used these methods and imaging mass spectrometry to directly examine plasticity that might lead to the formation of initial memory and a new approach with both intracellular and extracellular measurements at the same time has led to time resolved measurements of plasticity in real time. This plasticity is apparent as a change in fraction released which is observed in drug-treated cells.

We have compared electrochemical quantification of the molecules released during exocytosis and the vesicular molecular content in living neurons of the fruit fly. They show the fraction of molecules released from a vesicle during an exocytosis event is extremely small at 5 to 11%. Additionally, vesicle content is hundreds of thousands of molecules! A theoretical model to back-calculate the number of molecules in each vesicle verifies this controversial discovery.

METABOLIC CONTROL OF MEMORY FORMATION REVEALED BY 2-PHOTON IMAGING OF BRAIN ENERGY METABOLISM IN DROSOPHILA

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Memory, Energy metabolism, Drosophila, glia, metabolic imaging.

Essential brain functions, such as forming long-term memoy (LTM), acutely increase the energetic burden of implicated neuronal circuits, as shown in many species from Drosophila flies to humans. Thus, inability of meeting such fast demands results in pathological states that can range from loss of circuit coding precision to reduced survival. In neurons, mitochondria provide most of the required energy for neuronal function. The primary metabolite consumed by mitochondria is pyruvate, derived from glucose, which fuels the tricarboxylic acid cycle. Currently we miss a global picture of how mitochondria intervene in higher brain functions such as memory. Drosophila, despite having a much simpler brain compared to mammalian models, can feature elaborated memory processes involving well-described neuronal networks. Moreover, our lab has developed pioneer 2-photon imaging protocols to measure *in vivo* intracellular metabolic fluxes in neurons (1,2) using genetically-encoded FRET metabolic sensors. Using this model, we showed that an acute upregulation of mitochondrial pyruvate uptake within the fly's major memory center, the mushroom body (MB), is both necessary and sufficient to drive LTM formation (3). This establishes mitochondria as an unexpected critical regulatory checkpoint in the formation of LTM. But how do mitochondria exert such a control, and how is pyruvate provided to mitochondria for memory fueling?

Our recent results show that glial cells are essential in providing pyruvate to neurons, establishing in vivo the relevance of neuron-glia metabolic compartmentalization for a higher brain function. In addition, recent data showing how dopamine-signaling involved in memory consolidation modulates mitochondrial metabolism will be discussed.

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OPTIMISING FAST SCAN CYCLIC VOLTAMMETRY ANALYSIS FOR THE DETECTION OF DOPAMINE IN THE ZEBRAFISH BRAIN

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Keywords : Voltammetry, Dopamine, Zebrafish, MATLAB, Neurotransmitters

Detecting the release and reuptake of dopamine in the adult zebrafish (Danio rerio) brain presents unique challenges. The cyclic voltammogram (CV) is a visual representation of the oxidative and reductive profile of the electroactive substance released due to stimulation. Neurotransmitters such as dopamine have unique and recognisable CVs allowing them to be identified. Following electrical stimulation, optimised for the detection of dopamine, the adult zebrafish brain produces a CV that is dopamine like but appears to have other contributors to the profile. This work evaluated the ability of a calibration set of cyclic voltammograms at a variety of concentrations to resolve the neurochemical composition of solutions via principal component regression analysis. The constructed training set was evaluated for robustness and meta-analysis using rodent data was also conducted as a proof of principle step. The principal component regression resolved the changes in dopamine, pH, 5-HT, and histamine release evoked following electrical stimulation. The covarying nature of the code outputted concentrations relates to the electrically evoked nature of the neurotransmitter release, in essence a simulated environment. Further teasing out of the results in detail, particularly the response of dopamine compared to 5-HT in response to dopamine reuptake inhibitors allows further exploration of the robustness of this analysis method. The results of the analysis of histamine raises further questions about expanding and tailoring the calibration set depending on the biological model in question, however the results display that the resolution of overlapping cyclic voltammograms is possible with an appropriate calibration (training) set.

REGULATION OF SOMATODENDRITIC DOPAMINE RELEASE FROM THE INSIDE

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Keywords: substantia nigra, voltage-clamp recording, SNAP-25, synaptotagmin, calcium dependence

Somatodendritic release of dopamine (DA) acts at D2 autoreceptors to regulate DA neuron firing patterns, thereby influencing DA release throughout the brain. Although decades have passed since the discovery of somatodendritic DA release, only recently have tools become available to address fundamental questions about this process. Our recent work has used voltage-clamp recording of D2-receptor-activated K^+ currents in DA neurons in the substantia nigra pars compacta (SNc). These DA-dependent currents serve as biosensors of somatodendritic DA release. Using single-cell application of antibodies and toxins via recording pipettes, we identified several intracellular proteins involved in the release process. A fundamental finding is that a given SNc DA neuron is autoinhibited primarily by its own DA, not that released by neighbouring DA cells. Using intracellular antibodies and a SNAP-25-specific botulinum neurotoxin, we found that SNAP-25 plays a key role in somatodendritic DA release, confirming that release is exocytotic. We also found complementary roles for the intracellular Ca^{2+} sensors synaptotagmins 1 and 7 using this approach wild-type and knockout mice, and showed that synaptotagmin 7 mediates the high Ca^{2+} sensitivity of somatodendritic DA release. Together, these studies reveal the primary source of autoinhibitory DA, the exocytotic nature of the intracellular release process, and the Ca^{2+} sensing proteins that mediate different aspects of somatodendritic DA release.

TALKING TO ONE'S SELF: AUTOREGULATION OF STRIATAL DOPAMINE RELEASE BY CO-RELEASED GABA

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Keywords : Fast-scan cyclic voltammetry, $GABA_A$ receptors, GAT1, ex vivo slices

Striatal dopamine (DA) axons co-release GABA that is sequestered from the extracellular compartment via GAT1 transporters on DA axons and packaged in DA vesicles. However, the function of this phenomenon is unknown. Given that DA axonal D2Rs can autoinhibit DA release and that DA and GABA share the same vesicle, we tested whether co-released GABA also autoregulates DA release via $GABA_A$Rs on DA axons. We first confirmed the anatomical presence of $GABA_A$Rs on DA axons using immuno-electron microscopy and found that almost half of TH-positive axons in the dorsal striatum (dStr) and nucleus accumbens core (NAc) express α3-$GABA_A$R subunits. To test whether DA axonal $GABA_A$Rs are functional, we examined the effect of a $GABA_A$R agonist on optically-evoked increases in extracellular DA concentration ($[DA]_o$), monitored with fast-scan cyclic voltammetry in *ex vivo* slices from male and female Ai32:DAT-Cre mice. Activation of $GABA_A$Rs with muscimol decreased single-pulse (1 p) evoked $[DA]_o$ by ~25% in both dStr and NAc, confirming functionality. Conversely, the $GABA_A$R channel blocker, picrotoxin (PTX), increased 1 p optically evoked $[DA]_o$ in both regions consistent with an endogenous $GABA_A$R tone in striatal slices. We then used an optical pulse-train stimulation (10 p, 10 Hz) to assess the effect of co-released GABA on DA release during a stimulus mimicking phasic axonal activity. PTX increased pulse-train evoked $[DA]_o$ throughout the striatum in both sexes, but to a greater extent than seen with 1 p. This is consistent with inhibition of DA release by co-released GABA during subsequent pulses in the pulse-train. Moreover, PTX amplified the ratio of 10 p-to-1 p-evoked DA release indicating that GABA co-release normally dampens phasic-to-tonic DA signaling. Importantly, the differential effect of PTX on optically-evoked phasic-to-tonic DA signaling is lost in mice devoid of GAT1 in DA neurons that lack GABA co-release. These data provide evidence for autoinhibition of axonal DA release by co-released GABA that is likely faster than G-protein coupled D2 autoreceptors, and introduce α3-$GABA_A$Rs as potential mediators of this novel regulatory process.

CAN WE UTILISE ENOGENOUS REGULATORS OF L-TYPE CHANNELS AS NEUROPROTECTIVE STRATEGIES AGAINST PARKINSON'S

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Keywords : Dopamine, Parkinson's, Calcium channels, alpha-synuclein, striatum

Dopamine (DA) neurons of the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA), which project to dorsal and ventral striatum respectively differ in a number of ways. Notably in their high and low sensitivity to parkinsonian degeneration respectively. We have previously identified that DA release is differentially gated by L-type voltage-gated Ca^{2+} channels (LTCC) in the dorsal and ventral striatum. Given that LTCC function has been identified as a stressor of DA neurons at risk for parkinsonian degeneration we are interested in identifying the molecular mechanisms regulating LTCC function. Using fast-scan cyclic voltammetry in acute ex-vivo mouse brain to access mechanisms regulating LTCC control of DA release across striatal territories in both sexes. We identify that risk factors for Parkinson's, including alpha-synuclein, male sex and DA-transporter (DAT) promote LTCC control of DA release, whereas protective factors, including calbindin-D28K inhibit LTCC control. We also identify that targeting $\alpha_2\delta$ subunits with gabapentinoid drugs limits LTCC function without compromising DA release. These findings sought us to further investigate the neuroprotective and therapeutic potential of gabapentinoid drugs in the treatment of Parkinson's.

STRIATAL DOPAMINE AND HYDROGEN PEROXIDE TRANSIENTS ASSOCIATED WITH L-DOPA INDUCED ROTATION IN HEMIPARKINSONIAN RATS

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Keywords: fast-scan cyclic voltammetry, Parkinson's disease, oxidative stress, dyskinesia, IR-MALDESI Parkinson's disease (PD) is a neurodegenerative disorder commonly treated with levodopa (L-DOPA), which eventually induces abnormal involuntary movements (A.I.M.s) that can be as debilitating as the disease itself. These dyskinesias have been linked with aberrant activity in the striatal direct and indirect pathways; however, the neurochemical contributors to striatal dysfunction remain unclear. Excitatory glutamatergic activity in striatum is intricately linked with dopamine (DA) signaling to fine tune the activity of medium spiny neurons, which play a critical role in the functional expression of motor control. Notably, hydrogen peroxide (H_2O_2) is produced upon glutamatergic activation of ionotropic receptors. H_2O_2 reduces local DA release, but the relevance of this modulation on motor output remains unclear. In this work, fast-scan cyclic voltammetry (FSCV) was used to investigate these molecules in the context of movement using a unilaterally 6-OHDA-lesioned rat model treated chronically with L-DOPA. DA and H_2O_2 were simultaneously monitored at single recording sites in both hemispheres across the course of 3 weeks of L-DOPA treatment. Mass spectrometry imaging was used to quantify the relative extent of DA loss. Hemiparkinsonian rats exhibited classic L-DOPA-induced A.I.M.s and rotations. L-DOPA treated animals exhibited an overall increase in DA and H_2O_2 tone over saline controls after 1 week of treatment. At this time, examination of rapid neurochemical dynamics revealed no clear relationship between DA or H_2O_2 fluctuations and A.I.M.s (dyskinetic behavior), or the onset of rotation. By week 3, DA tone remained elevated beyond controls, but H_2O_2 tone was largely normalized. At this time point, rapid neurochemical transients were time-locked with spontaneous bouts of rotation. Striatal H_2O_2 rapidly increased with the initiation of contraversive rotational behaviors in lesioned L-DOPA animals, as DA concentrations at the same site simultaneously decreased. The results support a role for these striatal neuromodulators in the adaptive changes that occur with L-DOPA treatment in PD, and reveal a precise interplay between DA and H_2O_2 in the initiation of involuntary locomotion.

LONG-LASTING ALTERATIONS IN AXONAL DOPAMINE RELEASE REGULATION & UPSTREAM TRANSCRIPTION INDUCED BY ETHANOL DRINKING IN MACAQUES

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Keywords: Kappa opioid receptor, mesolimbic dopamine, fast scan cyclic voltammetry, ethanol, rhesus macaque

Though alcohol use disorder is prevalent across the globe, pharmacological treatments still have limited efficacy and relapse rates remain high. A critical component in the development of substance use disorders is experience-dependent plasticity in the mesolimbic dopamine system, which is composed of dopamine projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). The kappa opioid receptor (KOR) system is a dynamic regulator of dopamine release in the NAc, and KOR antagonism reliably shows therapeutic promise for alcohol use disorders. The prevailing theory of KOR involvement in substance use posits that experience-dependent upregulation of the KOR system decreases dopamine release, thus driving relapse after periods of abstinence. However, minimal work has been conducted in non-human primates – a critical next step for advancing therapeutic endpoints and furthering our understanding of primate neurophysiology. Here, we investigate the interplay of KOR activity and dopaminergic function in rhesus macaques following 18 months of ethanol self-administration and three 1-month forced abstinence periods. Following the final 1-month abstinence period, subjects were sacrificed for *ex vivo* fast-scan cyclic voltammetry recordings in NAc and RNA sequencing of VTA. In the NAc core, elicited dopamine release was increased by KOR antagonism to a greater extent in drinkers compared to controls, indicating augmented inhibitory feedback via release of endogenous dynorphins. Application of a KOR agonist revealed marked upregulation of KOR inhibition of dopamine release in drinkers, demonstrating that augmented KOR sensitivity persists well into abstinence. Paired with RNA-seq data from the VTA of the same animals, we define the upstream transcriptional signatures that may mediate the long-lasting alterations in KOR function. Together, this data demonstrates prolonged upregulation of KOR-mediated inhibition of dopamine release both through increased endogenous activity and response to exogenous ligand. This work brings a new understanding of circuit regulation and gene expression associated with voluntary alcohol use in a non-human primate model.

PROBING STRIATAL CIRCUITS WITH PHOTO-RELEASABLE AZOSOMES

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Keywords : azosomes, neuropeptide sensor, photo-release, volume transmission, brain

Neuropeptides are important neuromodulators in the brain, yet remarkably little is known about their spatiotemporal spread, action on neural circuits, and effect on behavior. To address this gap, our laboratories are developing photoswitchable nanovesicles that are activated with widely available LEDs and can be integrated with fluorescence-based neuropeptide sensors (e.g. CNiFERs, GRABs, dLights) and measurements of neuronal activity (e.g., GCaMP). Here, we describe some of our advances with this endeavor. We generated light-sensitive azobenzene-based liposomes ('azosomes') and first studied the photorelease of a fluorescent dye (i.e. calcein) in vivo with pulses of 365 nm light. We determined the stability and photo-release efficiency with liposome composition, cargo´s molecular weight and light pulse power/duration. We then used opto-fluidic cannulas that allow photometry measurements in vivo while also delivering a small modulator or azosomes-loaded with a modulator into the dorsal striatum. We monitored induced release, neuronal activity and locomotor activity in a freely-behaving mice. Flex-GCaMP7s AAV virus was first injected into dorsal striatum of D1-Cre mice to monitor activity of direct (D1) medium spiny neurons. After 2-3 weeks, photometry measurements were made while simultaneously photo-releasing either a D1-MSN activator (SKF81297) or D1 inhibitor (Adenosine A1 receptor agonist N6-Cyclopentyladenosine) and monitoring D1 MSN activity in vivo and behavior. Preliminary results showed that local release of CPA silences D1-MSNs and reduces locomotor activity. These newly developed techniques will advance our understanding of the role of neuromodulators in the brain and more broadly, promote new neuropharmacology research where targeted delivery and localized release of a compound are currently unavailable.

SEEING THE SOUND: OPTICAL AND ULTRASONIC INTERFACES FOR NEUROMODULATION

Xiang Wu,[1,2] Nicholas J Rommelfanger,[1,3] Zihao Ou,[1,2] Shan Jiang,[1,2] Guosong Hong[1,2] (Arial 9 Bold)

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Keywords : optogenetics, infrared, ultrasound, stimulation, imaging

Today's optical neuromodulation methods enable causal manipulation of neural activity with light to dissect complex circuit connections underlying certain behaviors. In these optical neuromodulation approaches, visible light with wavelengths between 430 nm and 640 nm is commonly used, thus limiting penetration depth in vivo and resulting in an invasive fiber-tethered interface that damages the endogenous neural tissue and constrains the animal's free behavior. In this talk, I will present three recent methods to address this challenge: sono-optogenetics, infrared optogenetics, and in vivo tissue transparentization enabled by the Kramers-Kronig relation. In sono-optogenetics, we demonstrate that mechanoluminescent nanoparticles can act as a systemic light source to convert focused ultrasound into localized light emission for noninvasive optogenetic neuromodulation in live mice. In infrared optogenetics, we demonstrate 1064-nm near-infrared-II light can enable tether-free and implant-free neuromodulation throughout the entire brain in freely behaving mice. Lastly, we demonstrate that the Kramers-Kronig relation can be used to yield tissue transparency in live animals, thus facilitating deep-tissue penetration of visible light for optical imaging. I will conclude my talk by presenting an outlook on how these approaches may advance neuroscience research in live animals and even humans.

IMAGING NEUROMODULATORS IN THE BRAIN WITH NEAR-INFRARED FLUORESCENT NANOSENSORS

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Keywords : Neuromodulation, near-infrared imaging, nanomaterials, dopamine, serotonin

Neurons communicate through neurotransmitter signals that either terminate at the postsynaptic process (“wired transmission”) or diffuse beyond the synaptic cleft to modulate the activity of larger neuronal networks (“volume transmission”). Molecules such as dopamine, serotonin, and neuropeptides such as oxytocin belong to the latter class of neurotransmitters and have been the pharmacological targets of antidepressants and antipsychotics for decades. Owing to the central role of neuromodulators over a range of behaviors and psychiatric disorders, real-time imaging of the signal’s spatial propagation would constitute a valuable advance in neurochemical imaging. To this end, we present a library of nanoscale near-infrared fluorescent nanosensors for dopamine (Beyene et al. Science Advances 2019; Yang et al. Nature Protocols 2021), serotonin (Jeong et al. Science Advances 2019), and oxytocin, where the nanosensors are developed from polymers pinned to the surface of single wall carbon nanotubes (SWNT). We characterize our findings in the context of their utility for high spatial and temporal neuromodulator imaging in the brain, describe nanosensor exciton behavior from a molecular dynamics (MD) perspective, and validate nanosensor for use to elucidate neuromodulator signaling variability with disease or pharmacological perturbations at a synaptic scale. We next use this dopamine imaging nanosensor to study dopamine signaling deficits in Huntington’s Disease (HD), where dysregulation of dopamine transmission plays a key role in multiple neurodegenerative diseases. While several treatments for physical and psychiatric HD symptoms target dopaminergic neuromodulation, little is known about the relationship between dopamine and the principal cause of HD, production of mutant huntingtin protein. Specifically, knowledge of what drives decreased dopamine release at motor symptom onset is uncertain and could be driven by decreasing dopamine release site numbers, decreasing dopamine quantal release per site, or a combination of the two. By imaging dopamine activity in the striatum of R6/2 HD model mice, we find that late-disease decreases in evoked dopamine release are primarily driven by decreases in the number of dopamine release sites as opposed to net decreases in dopamine release per release site. We discuss how to use these findings as optimal therapeutic intervention timepoints for siRNA-based HD therapies, discuss how dopaminergic projections are affected by mutant huntingtin, and whether specific targeting of these loci is important for developing gene-therapy efforts.

PROBING NEUROPEPTIDE VOLUME TRANSMISSION IN VIVO BY SIMULTANEOUS NEAR-INFRARED LIGHT RELEASE AND OPTICAL SENSING

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Keywords: Plasmonic nanovesicles, neuropeptide sensor, photo-release, volume transmission, brain

Neuropeptides are essential signaling molecules in the nervous system involved in modulating neural circuits and behavior. Neuropeptides can diffuse over a large distance from the axons and act on the target through G-protein coupled receptors (GPCRs), which is referred to as volume transmission. Although neuropeptides volume transmission is critical to their functions, remarkably little is known about their extrasynaptic transmission. Here, we developed an optical approach to probe neuropeptide volume transmission in mouse neocortex. To control neuropeptide release, we engineered plasmonic nanovesicles (Au-nV-SST) that can release somatostatin-14 (SST) with near-infrared light stimulation. To detect the functional response of SST, we created a nM sensitive cell-based neurotransmitter fluorescent engineered reporter (CNiFER) using the SST2 GPCRs. Using the combination of Plasmonic nAnovesicles and CNiFERs (PACE), we designed an integrated system to mimic neuropeptide volume transmission, including the release, diffusion, binding and signaling. Under intravital two-photon imaging, we measured the time for the released SST to activate SST2 GPCRs and subsequent signaling at defined distances. This measurement reveals synchronous transmission within 130 μm while a delayed transmission at larger distances. Our study provides the first quantitative estimation of the overall SST loss rate due to the degradation and binding in vivo, and demonstrates that neuropeptide binding and degradation limits its extrasynaptic transmission at large distances (>100 μm). We further demonstrated that SST transmission is significantly faster in neocortex with a chemically degraded extracellular matrix. The PACE technique reveals the spatiotemporal scales of neuropeptide volume transmission and signaling in the brain and provides a useful tool to investigate important physiological processes in living systems.

LIGHT STIMULATION REDUCTION IN NEURONAL ACTIVITY IS DEPENDENT ON THE TEMPORAL PATTERN AND LIGHT POWER USED

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Keywords: Electrophysiology, optogenetics, neuronal activity

Neuronal sensitivity to light stimulation can represent a serious confounding factor for those assays using light to investigate neuronal processes such as optogenetics and optical imaging. Here we studied the effects produced by ten different patterns of light stimulation delivered to different populations of brain neurons. We showed that light in the visible spectrum produces an outward hyperpolarizing current and an inhibition of neuronal activity, which increases with the light duty cycle, pulse duration and power used. Importantly, these effects are correlated with the light-induced increase in tissue temperature. Overall our results provide a guideline to avoid artefactual effects when applying experimental protocols based on brain light stimulation.

INTEGRATED NANOSIMS AND FLUORESCENCE MICROSCOPY ANALYSIS OF NEURONAL CELLS

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Keywords: NanoSIMS, fluorescence, synapse, neuron, super-resolution.

Aged proteins must be replaced by newly produced ones in a precisely coordinated fashion, to avoid the accumulation of damaged molecules. This process, termed protein turnover, is especially important in neurons, since they are long-lived and are very difficult to replace, which implies that their metabolism, and the turnover of their different proteins and organelles, must be optimally regulated. This type of regulation is even more important at the synapse, since this compartment responds to two types of challenges: 1) turnover: as for all other cellular structures, the synapse needs to replace damaged proteins and organelles; 2) plasticity: the synapse needs to change dynamically its composition upon changes in network activity.
However, while individual protein lifetimes have been calculated for neuronal tissues, what actually happens on the level of individual synapses and organelles remains unclear. To address this issue, one needs to visualize (image) protein turnover, at the single organelle level. Typically, turnover can be investigated by pulsing the cells with isotopically-labelled precursors (such as amino acids). In addition, two conditions should be fulfilled: 1) the organelle needs to be clearly identified, and 2) the marker molecules that the cell was pulsed with must be visualized with high spatial resolution, to allow the precise measurement of turnover within the identified organelle. This is optimally achieved by combining nanoscale secondary ion mass spectrometry (nanoSIMS) with super-resolution fluorescence imaging. We used this correlative analysis procedure to describe multiple parameters, from synaptic vesicle turnover and synaptic function to structural aspects of synaptic plasticity. Importantly, these technologies can be fine-tuned to enhance resolution towards the single-nm level, and should also be applicable to many other biological questions.

QUANTITATIVE NANO-AMPEROMETRIC MEASUREMENT AND OF SUB-QUANTAL GLUTAMATE RELEASE BY LIVING NEURONS

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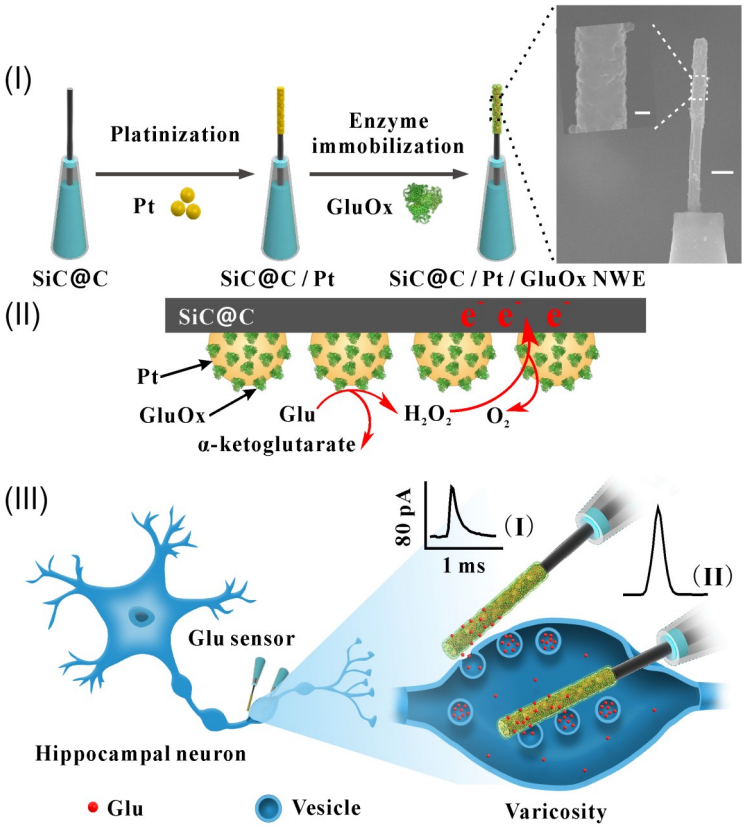
Keywords: glutamate (Glu) , single nanowire electrochemical sensor , vesicular Glu content , sub-quantal release , release fraction

Glutamate (Glu) is a crucial fundamental excitatory neurotransmitter released through vesicular exocytosis in the central nervous system. Hence, quantitative measurements and interpretation of intravesicular Glu and of transient exocytotic release contents directly from individual living neurons are highly desired for understanding the mechanisms (full or sub-quantal release?) of synaptic transmission and plasticity. However, this could not be achieved so far due to the lack of adequate experimental strategies relying on selective and sensitive Glu nanosensors.

We will show that a novel electrochemical Glu nanobiosensor based on a single SiC nanowire (I,II) is prone to selectively measure in real-time Glu fluxes released via exocytosis by large Glu vesicles (ca. 125 nm diameter) present in single hippocampal axonal varicosities as well as their intravesicular content before exocytosis by IVIEC (III).



Combination of these two series of measurements revealed a sub-quantal release mode in living hippocampal neurons, viz., only ca. one third to one half of intravesicular Glu molecules are released by individual vesicles during exocytotic events.

Importantly, this fraction remained practically the same when hippocampal neurons were pretreated with L-Glu-precursor L-glutamine, while it significantly increased after zinc treatment, although in both cases the intravesicular contents before release were drastically affected.

This work will also serve to discuss the meaning and adequacy of pre-calibrations performed in bulk solutions to assess the analytical properties of enzyme-based electrochemical nanosensors aimed to detect fast transient release events.

IMAGING OF LIPIDS AND PROTEINS IN NERVE CELLS WITH SIMS – FROM NON-TARGETED TO TARGETED BIOIMAGING

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Keywords: SIMS, bioimaging, nerve cells, molecular organization, turnover

Biological molecules have been shown to exhibit specific cellular localizations, which relate closely to their functions. We applied mass spectrometry imaging methods including time-of-flight secondary ion mass spectrometry (ToF-SIMS) and nanoscale SIMS (NanoSIMS) imaging to investigate the organisation of lipids and proteins and their dynamic turnover in nerve cells (hippocampal neurons, neural progenitor cells), and to relate these chemical structures to neuronal activity.

Different lipid species have been shown to specifically localize in different regions of nerve cells, particularly the cell body and neurites. This lipid organization was significantly altered following altered neuronal activity. In addition, specific patterns of molecular turnover have been observed at the neuronal plasma membrane and at single organelles, which indicates the regulatory roles of molecular turnover in cellular processes.

We have developed new chemical labels with both stable isotopic and fluorescent probes for correlative imaging with SIMS. This allows simultaneous chemical detection and correlation of targeted cellular structures in nerve cells at subcellular resolution. Newly synthesized probes together with correlative imaging approach provide a better understanding of the chemical organization in the nerve cells, and their involvement in different neuronal functions.

CLUSTERS OR CONDENSATES ? HOW ARC REGULATES AMPA RECEPTORS LEVEL

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Keywords : Arc, AMPA receptor, oligomerization, liquid-liquid phase separation, membrane binding

The Activity Regulated-Cytoskeleton associated-protein Arc is pivotal to mediate plastic responses in neuronal cells. In vitro studies suggest its ability to form large and small order oligomers which are potentially involved in interneuronal trafficking. Despite its important function, no direct observation of Arc oligomers in cells has been presented due to the small size, and lack of appropriate labelling strategies.
Here, we take advantage of STED microscopy to study Arc nanoscale organization in cellular environment especially at the synapses. Arc oligomers role in the regulation of AMPA receptor surface levels, together with their close association to the plasma membrane, were addressed via chemical mutagenesis and molecular dynamic simulation studies. Furthermore, for the first time, Arc-Arc molecular interaction and its liquid-liquid phase separation properties were uncovered in cellular system.
Together, our observations support the model by which Arc oligomerization at the post-synaptic endocytic zone, favors AMPA receptors endocytosis inducing

COMBINING TEM AND NANOSIMS IMAGING TO DISCERN VESICLE COMPARTMENTS IN PC12 CELLS AND QUANTIFY VESICULAR ISOTOPIC DOPAMINE CONCENTRATION

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Keywords: NanoSIMS, TEM, dense core vesicle, dopamine

The absolute concentration and the compartmentalization of analytes in cells and organelles are crucial parameters in the development of drugs and drug delivery systems, as well as in the fundamental understanding of many cellular processes. Nanoscale secondary ion mass spectrometry (NanoSIMS) imaging is a powerful technique which allows subcellular localization of chemical species with high spatial and mass resolution, and high sensitivity.For this project, NanoSIMS imaging and transmission electron microscopy (TEM) imaging were combined to discern the compartments (dense core and halo) of large dense core vesicles (LDCVs) in PC12 cells and to localize ^{13}C dopamine enrichment following ^{13}C L-DOPA incubation. LDCVs are easily recognized in TEM images owing to their characteristic dense core structure. However, from a $^{12}C^{14}N$ NanoSIMS image alone, is it not possible to distinguish them from other cellular organelles and cytoplasmic features where the amount of $^{12}C^{14}N$ naturally differs. Here, by overlaying TEM images and the highly resolved $^{12}C^{14}N$ secondary ion images, different vesicle compartments could be localized and appeared as distinct based on their $^{12}C^{14}N$ content. In addition, the absolute concentrations of ^{13}C dopamine in these vesicle domains as well as in entire single vesicles were quantified and validated by comparing with electrochemical data. This confirms that NanoSIMS imaging can be used to carry out absolute quantification within highly resolved, subcellular biological compartments. This approach adds to the potential of using combined TEM and NanoSIMS imaging to perform absolute quantification and directly measure the individual contents of nanometer-scale organelles.

HOW PET IMAGING IS ADVANCING THE DISCOVERY OF NEW CNS DRUGS

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Keywords: Neuropharmacology; Neurology; Psychiatry; Drug discovery; Radiopharmaceutical

The discovery of new drugs for the CNS remains a major challenge for neurology, and more particularly for psychiatry. The vast majority of psychotropic drugs were invented more than half a century ago and have sometimes limited efficacy and/or significant side effects. However, the research and development of new drugs remains complex with a very limited pipeline of candidate-molecules. Many failures are attributable to the pharmacodynamic/pharmacokinetic characteristics of the molecules, which have not been sufficiently explored prior to studies in humans.
PET imaging allows to explore these molecular and functional characteristics at preclinical and early clinical phases with non-invasive and translational in vivo approaches in both animal and human models. The different steps of brain drug mechanisms can be explored with PET imaging and specific radiotracers: the visualization of the pharmacological target (receptor, transporter, ...), the brain passage of the molecule, its engagement on the target, its target occupancy rate, its duration of action and the progression of its pharmacological effects. Thus, PET imaging studies adapted to each stage of drug development provide crucial information contributing to the proof of concept of the future CNS molecules.

DESIGN AND RADIOLABELING STRATEGIES FOR BRAIN PET RADIOTRACERS

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Keywords : radiochemistry, fluorine-18, carbon-11, PET radiotracers, lipophilicity

PET imaging is a useful tool for pharmacological studies, drug design and medical diagnosis. These last years the application of this tool to explore brain was well developed and illustrated.
However, such medical imaging requires the use of radiotracers to visualize the target. These radiotracers are small molecules, peptides or antibodies which must be radiolabelled with a positron-emitter radioisotope.
The most used radioisotopes are mainly fluorine-18 and carbon-11. Nevertheless, their introduction onto targeted molecules (or macromolecules) requires specific strategies to obtain the expected compounds.
Furthermore, the brain targeting implies additional constrains to the molecules which should cross the blood brain barrier (BBB).
The properties required to facilitate the BBB permeation will be discussed. The labelling strategies to easily radiolabel the various molecules and macromolecules will be also described.

PET IMAGING EXPLORATION DOPAMINE AND SEROTONIN TRANSMISSIONS IN PSYCHIATRIC DISORDERS OF PARKINSON'S DISEASE

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Keywords : Apathy, Depression, Anxiety, impulse control disorders, multiple-tracers PET study, patients and primate model with double lesions

Parkinson's disease (PD) is characterized by heterogeneous motor and non-motor symptoms related to alterations in monoaminergic systems. The characterization of the dopaminergic (DA) and serotonergic (5-HT) dysfunctions after different durations of Parkinson's disease, as well as their respective involvement in the expression and severity of neuropsychiatric signs, like apathy, depression and anxiety, has gained little attention so far. To fill this gap, we conducted several studies focusing on positron emission tomography (PET) imaging with several radiotracers of DA and 5-HT transmission, in patients at different stages of PD, as well as on the non-human primate (NHP) with double lesions of the DA and 5-HT systems induced by injections of MPTP and MDMA (Ecstasy). The results of these studies highlight the interest of two radiotracers, the [11C]-PE2I and the [11C]-DASB, which bind respectively the DA and 5-HT reuptake transporters. These radiotracers allowed us to determine the lesion profile linked to the appearance of depression, apathetic state and the anxiety trait as well as the cerebral structures targeted by these two monoaminergic systems and involved in the expression of these psychiatric symptoms. These two radiotracers have also highlighted the importance of the 5-HT innervation on corticostriatal circuits linking the ventromedial frontal cortex and the anterior insular to the ventral striatum in the expression of neuropsychiatric symptoms but also to the resistance of PD patients to SSRI treatments. Finally, this work also underlines the importance to develop new molecules that can replace SSRI treatments whose resistance is frequent (around 30% of patients) in many psychiatric disorders. Our new primate model with 5-HT lesion induced by MDMA coupled with PET imaging of multiple 5-HT radiotracers could be highly useful for these therapeutic developments.

PET IMAGING CONTRIBUTIONS TO THE UNDERSTANDING OF NEURODEGENERATIVE PATHOLOGIES

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Keywords : PET, biomarkers, synaptopathy

New concept are emerging for understanding neurodegenerative pathologies based on biomarkers. Biomarkers characteristic of a brain pathology allow us to consider preclinical stages of a disease. It is then possible to track different stages and to try understanding the most relevant features leading to clinical symptoms. The classification of Alzheimer's disease into Amyloid, Tau and Neurodegenerative features is such an example. New radiotracers for Positron Emission Tomography allow to measure synaptopathy as an early type of neurodegeneration.

PRECLINICAL PET/FMRI IMAGING OF A BIASED AGONIST OF SEROTONIN RECEPTORS IN A RAT MODEL OF L-DOPA-INDUCED DYSKINESIA

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Keywords: functional imaging; Parkinson's disease; serotonin; Levodopa-induced dyskinesia; biased agonist

The leading treatment for motor symptoms of Parkinson's disease is L-DOPA, but, upon extended use, it can lead to side effects like levodopa-induced dyskinesia (LID). Serotonergic neurons are involved in the etiology of LID and previous pre-clinical studies have shown that NLX-112, a 5-HT_{1A} receptor biased agonist, has robust antidyskinetic effects[1]. The aim of this study was to investigate its effects in hemiparkinsonian (HPK) rats with a unilateral nigrostriatal 6-OHDA lesion with or without chronic L-DOPA administration to elicit LID, using functional imaging.

We compared HPK rats with LID (i.e. sensitized to the dyskinetic effects of chronic L-DOPA) and without LID (HPK-non-LID), using [^{18}F]FDG PET imaging and fMRI functional connectivity following systemic treatment with saline, L-DOPA, NLX-112 or L-DOPA+NLX-112. In HPK-non-LID rats, [^{18}F]FDG PET experiments showed that L-DOPA led to hypermetabolism in motor areas (cerebellum, brainstem, and mesencephalic locomotor region) and to hypometabolism in cortical regions. These effects of L-DOPA were also observed in HPK-LID rats, with the additional emergence of hypermetabolism in raphe nuclei and hypometabolism in hippocampus and striatum. NLX-112 attenuated the raphe hypermetabolism and cingulate cortex hypometabolism induced by L-DOPA in HPK-LID rats. Moreover, in fMRI experiments NLX-112 partially corrected the altered neural circuit connectivity profile in HPK-LID rats, through activity in regions rich in 5-HT_{1A} receptors. This neuroimaging study sheds light for the first time on the brain activation patterns of HPK-LID rats. The 5-HT_{1A} receptor agonist, NLX-112, prevents occurrence of LID, likely by activating pre-synaptic autoreceptors in the raphe nuclei, resulting in a partial restoration of brain metabolic and connectivity profiles. In addition, NLX-112 also rescues L-DOPA-induced deficits in cortical activation, suggesting potential benefit against non-motor symptoms of Parkinson's disease.

Reference:

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DYNAMICS AND MODULATION OF STRIATAL ACETYLCHOLINE

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Keywords: Basal Ganglia, Genetically-encoded Sensors, Photometry, Neuromodulation, Cholinergic Interneurons

Acetylcholine (ACh) has critical roles in striatal function and related behaviors. The majority of striatal ACh is supplied by the cholinergic interneurons (ChIs) in striatum, but afferents from extrastriatal sources also contribute. To examine dynamics and modulation of striatal ACh release we expressed the genetically-encoded ACh sensors iAChSnFR and GRABACh3.0 by injecting AAV constructs intrastriatally. Brain slice photometric recordings from dorsomedial striatum showed that single-pulse intrastriatal electrical stimulation produces robust ACh release measured with either sensor. These stimulation-induced increases were dependent on neuronal firing and extracellular calcium, but did not require activation of fast glutamatergic transmission. Surprisingly, fluorescence increases measured with iAChSnFR persisted for 10s of seconds while those measured with GRABACh3.0 persisted for a few seconds. The prolonged increases measured with iAChSnFR were observed with microstimulation and optical activation of ChIs with the Chrimson opsin. Inhibition of the vesicular ACh transporter or lesioning of ChIs greatly reduced the prolonged increases observed with iAChSnFR. The prolonged responses measured with iAChSnFR may be driven by increases in choline, as this sensor has appreciable choline sensitivity. Small amplitude spontaneous fluorescence increases were observed with both sensors, and these transients persisted for a few sec or less with either sensor. ACh release measured with either sensor is suppressed by activation of D2-like dopamine receptors. Activation of M2/M4-type muscarinic ACh receptors (mAChRs) also suppresses ACh release. Interestingly, electrical stimulation-induced ACh activates M1 mAChRs that contribute to increases in the endocannabinoid 2-arachidonoyl-glycerol, as measured with the genetically-encoded GRABeCB sensor. In vivo photometry experiments indicate that we can measure changes in striatal ACh induced by environmental stimuli, cocaine and alcohol using iAChSnFR. We are assessing how changes in ACh contribute to behaviors involving the dorsal striatum.

IMAGING SIGNALING EVENTS IN RESPONE TO NEUROMODULATORS AT SINGLE CELL RESOLUTION *IN VIVO*

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Keywords : neuromodulation; in vivo imaging; protein kinase A (PKA); cyclic AMP (cAMP)

Slow synaptic transmission, also known as neuromodulation, plays pivotal roles in regulating diverse brain functions, and imposes powerful control over the function of fast synaptic transmission. Neuromodulators function by cell-specific regulation of intracellular signalling pathways. Therefore, monitoring these signalling dynamics *in vivo* with single neuron resolution is an essential component of investigating brain functions. Notably, major neuromodulators, such as dopamine, norepinephrine, acetylcholine, and serotonin, can all signal through the cAMP/PKA pathway. This pathway, in turn, regulates neuronal excitability, synaptic transmission, and metaplasticity. We will present our recent work of developing, implementing, and thoroughly characterizing the new-generation of genetically encoded cAMP and PKA sensors for *in vivo* imaging at single cell and subcellular resolution. Furthermore, we will discuss our findings utilizing these sensors as a readout for cell type-specific responses to multiple neuromodulators, including dopamine, norepinephrine and adenosine in the behavioural contexts, as well as cellular and circuit responses to opioid modulation.

LOCAL NEURAL MECHANISMS OF THERAPEUTIC DEEP BRAIN STIMULATION IN PARKINSON'S DISEASE

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Keywords : deep brain stimulation, Parkinson's disease, genetically encoded fluorescent indicators, chemogenetic

Deep brain stimulation (DBS) is currently the most effective treatment for alleviating motor symptoms in patients with advanced Parkinson's Disease (PD). However, the mechanisms underlying its therapeutic effects remain elusive. Here we use genetically encoded fluorescent sensors to show that therapeutic DBS in the subthalamic nucleus (STN), the most common target for treating PD, induces persistent activation in the afferent axon terminals while inhibiting postsynaptic neurons in the STN. These differential effects on pre- and postsynaptic activities are likely caused by the depletion of synaptic vesicles because prolonged DBS causes a decrease in the levels of local neurotransmitters in the STN. Based on these results, we hypothesize that chemogenetic inhibition of STN neurons should achieve the same therapeutic outcomes as DBS. We use viral vectors to express inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) hM4Di in the STN of MitoPark mice, a genetic PD model with age-dependent loss of dopaminergic neurons, and show that DREADD agonist Clozapine N-oxide (CNO) inhibits STN neurons and rescues the motor deficits in these mice. These findings elucidate the neural mechanisms of STN DBS, and point to a promising chemogenetic gene-therapy treatment that is less invasive, more affordable for treating advanced PD.

DEFICIENCY IN ENDOCANNABINOID SYNTHASE *DAGLB* CONTRIBUTES TO PARKINSON'S DISEASE AND DOPAMINERGIC NEURON DYSFUNCTION

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Keywords: Parkinson's disease, dopaminergic neurons, endocannabinoid, diacylglycerol lipase b, 2-arachidonoyl-glycerol

(max. 2200 characters including spaces): Endocannabinoid (eCB) 2-arachidonoyl-glycerol (2-AG), the most abundant eCB in the brain, regulates diverse neural functions. Like dopamine, eCB signaling is also altered in Parkinson's disease (PD), the most common degenerative movement disorder. However, whether the observed eCB changes are a cause or compensatory response of the disease remains unclear. Using homozygosity mapping and whole-exome sequencing, we linked multiple homozygous loss -of-function mutations in diacylglycerol lipase b (*DAGLB*) to a form of early-onset autosomal recessive PD. We then used RNA sequencing and fiber photometry with genetically encoded eCB sensors to demonstrate that DAGLB is the main synthase of 2-AG in nigral dopaminergic neurons (DANs). In mice, the nigral 2-AG levels were markedly correlated with the motor performance during motor skill acquisition. Genetic knockdown of *Daglb* in nigral DANs substantially reduced nigral 2-AG levels and impaired motor skill learning, particularly the across-session learning, whereas pharmacological inhibition of 2-AG degradation increased nigral 2-AG levels, DAN activity and dopamine release and rescued the motor skill learning deficits. Together, we demonstrate that *DAGLB*-deficiency contributes to the pathogenesis of PD, reveal the importance of DAGLB-mediated 2-AG biosynthesis in nigral DANs in regulating neural activity and dopamine release, and provide preclinical evidence for the beneficial effects of 2-AG augmentation in PD treatment.

NUTRIENT-SPECIFIC APPETITES REVEALED BY IN VIVO FIBRE PHOTOMETRY

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Keywords : protein restriction, dopamine, mesolimbic circuits, calcium sensor

Acquiring the necessary balance of nutrients in one's diet is a compelling problem faced by many animals including humans. Lack of certain nutrients leads to profound changes in physiology and behaviour aimed at compensating for the deficiency. For the macronutrient protein, we have shown that when rats or mice are maintained on a low-protein diet (5% vs. 20%), they develop a preference for the dairy protein, casein, over the carbohydrate, maltodextrin. This preference is associated with increased palatability of protein, relative to carbohydrate, as measured by lick microstructure. In addition, protein-restricted rats appear more motivated for protein-rich food, as evidenced by elevated breakpoints in tests of progressive ratio responding. To determine neural circuits that are involved in these behavioural changes we have used in vivo fibre photometry. Ventral tegmental area neurons were targeted with a non-specific AAV to express the calcium sensor, GCaMP6s. Increased emitted fluorescence of the sensor acted as a proxy for neural activity. We found peaks in neural activity in all rats upon presentation of sippers containing isocaloric nutrient solutions and once they started drinking from the sippers. In non-restricted control rats, neural responses to protein (4% casein) and to carbohydrate (4% maltodextrin) were of similar magnitude whereas in protein-restricted rats responses to protein were greater than to maltodextrin. When diets were switched so that previously non-restricted rats became protein-restricted and vice versa, we found that newly protein-restricted rats rapidly developed preference for protein and this was associated with increased neural activity. In previously protein-restricted rats that had protein levels restored, behavioural and neural responses to protein were more persistent and did not rapidly adapt. Ongoing studies are further investigating this phenomenon using the dopamine sensor, GRAB-DA2m, to measure dopamine release in nucleus accumbens lateral shell.

BRAIN GLYCOGEN STORES RECRUITMENT DURING SPREADING DEPOLARIZATIONS EVIDENCED BY MICROELECTRODE BIOSENSORS

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Keywords: amperometry, rat, lactate, glucose, oxygen, metabolism

Brain metabolism can be monitored in vivo using microelectrode biosensors providing second by second estimates of glucose, lactate and oxygen concentrations. We have developed minimally invasive probes with only 12-15 µm diameter, using platinized carbon fibers, that avoid damaging blood vessels. These devices can monitor dynamic changes in brain metabolism in response to spreading depolarization, a propagating wave of near-complete depolarization of neurons and glial cells representing a physiological challenge for the brain tissue. We used this stimulus to investigate the role of glycogen stores in the regulation of brain energy metabolism. Brain energy stores consist mostly in glycogen present in astrocytes. They are recruited in ischemic conditions but also during episodes of high neuronal activity during normal brain functioning. The time course of this recruitment and its consequence on brain physiology is largely unknown.

We studied the consequences of blocking glycogen store recruitment with 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) an inhibitor of glycogen phosphorylase, on brain lactate, glucose, and oxygen concentrations using enzymatic microelectrode biosensors. Saline or DAB was injected at the site of biosensor and electrocorticogram recordings to block glycogen phosphorylase 1h before CSD induction.

Blocking glycogen stores recruitment increased the duration of tissue depolarization during SDs from 25 ±1.3 s to 34 ±1.3 s ($p<0.001$, $n=12$). By contrast the duration of the spreading depression of activity was left unchanged (211 ±41 s control, 248 ± 18 s DAB, $p=0.25$). SDs induced a transient decrease in cortical extracellular glucose concentration accompanied by lactate release. In the presence of DAB, the glucose decrease lasted longer (88 ±15 s control, 176 ±21 s DAB, $p=0.001$, $n=7$), and lactate release was diminished from 2006 ±700 µM to 1159 ±171 µM after DAB ($p=0.047$, $n=7$). In addition, changes in brain tissue oxygen pressure were similar in the presence or absence of DAB. We conclude that glycogen stores can be recruited within seconds to provide a boost in energy metabolism and lactate release into the interstitial fluid.

EXPLORING SURFACE-ENHANCED RAMAN SCATTERING OPTOPHYSIOLOGY FOR MAKING SENSE OF COMPLEX BRAIN NEUROCHEMISTRY

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Keywords: Raman spectroscopy, dopamine, glutamate, cultured neurons, mice

Brain neurochemistry is complex and a growing literature reveals that many neurons use more than one type of chemical messenger as neurotransmitters. As an example, neurons in the brain previously classified as “dopaminergic” often have the capacity to release not only dopamine, but also glutamate, GABA and multiple neuropeptides. To add to the complexity, in brain diseases such as Parkinson’s, the neurotransmitter repertoire of neurons is increasingly recognized as showing adaptations. Together, this makes it challenging to decipher the mechanisms at play in brain diseases and to identify and monitor the efficacy of new therapeutic targets. Although classic electrophysiological and neurochemical techniques, together with optical imaging and genetically encoded sensors are still important and powerful to analyse the functionality of brain circuits, there is an unmet need for new approaches that allow the dynamic measurement of multiple neurotransmitters at the same time in specific brain regions. Surface-enhanced Raman scattering (SERS) optophysiology is a new approach that we are developing to achieve this goal. Although still at its first steps, we have recently been able to demonstrate the potential of this approach for multiplex detection of neurotransmitters using cultured neuron preparations. This presentation will describe some of these initial efforts and the challenges that we face to translate this approach to more intact tissues such as brain slices. Work supported by the Fonds de Recherche du Québec, Nature et Technologie.

RECENT DEVELOPMENTS IN INTENSITY-BASED NEUROTRANSMITTER AND METABOLITE-SENSING FLUORESCENT REPORTERS

Jonathan S Marvin[1], Abhi Aggarwal[1,3], Phillip M Borden[1,4], Timothy A Brown, Jeremy Hasseman[1,2], Ilya Kolb [1,2], Loren L Looger[6], Kaspar Podgorski[1,3], Ariana N Tkachuk[1,5]

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Keywords : glutamate, GABA, ATP, fluorescent sensor, acetylcholine

True understanding of neuronal signaling and computation requires the ability to detect – and ideally visualize – specific neurotransmitters and neuromodulators in action. This is enabled by the relatively recent development of genetically encoded fluorescent neurotransmitter sensors. The most extensively optimized and widely used set of sensors is the GCaMP series of calcium indicators, constructed from the calcium binding protein calmodulin and circularly permuted GFP. We have developed several fluorescent sensors, which incorporate a GCaMP-like signaling mechanism, but are specific, with appropriate binding affinity, for different neuronal signaling molecules: glutamate, GABA, acetylcholine, and ATP. I will discuss recent, unpublished advances in our development of these sensors and preliminary results obtained from research groups validating them in multiple in vivo preparations.

Specifically, iGluSnFR3 is a marked improvement over the previous two generations of iGluSnFR and is useful for the detection of small and rapid synaptic glutamate transients. iGABASnFR and iGABASnFR2 reliably report GABA release in multiple in vivo models, including axonal release from individual interneuron boutons. iAChSnFR reports acetylcholine release in a variety of mouse and Drosophila circuits. Finally, we have developed an ATP sensor that allows visualization of ATP from individual release sites in primary neuronal culture.

Furthermore, we have also developed sensors for monitoring metabolites including glucose, aspartate, and cytoplasmic ATP.

FIBER-BASED BIOSENSORS AND MICROFLUIDICS FOR MONITORING TRANSIENT NEUROCHEMICALS

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Keywords: fibres, biosensors, lactate, dopamine

Understanding disease states requires both acute and chronic chemical monitoring within the body. Perhaps the clearest examples are clinical blood tests, which provide rich data at a single time point at the cost of long analysis times; and blood glucose monitoring, where concentrations of a single chemical are collected at multiple time points. The crucial next step is real-time, accurate chemical monitoring of a broader range of analytes. Focusing on the brain, tools able to monitor transient neurochemical dynamics are required in order to decipher brain chemistry and function.1 Traditionally, microdialysis and implantable electrodes have been used to monitor fluctuating neurochemistry. Devices introducing the multifunctionality of fluidics and electrodes are a strategy that adds capability. A thermal fibre-draw process enables the fabrication of complicated flexible fibres at scale. 2 We are examining hybrid fibres that combine aspects of both microdialysis and electrochemical sensors. The presentation will describe the design and construction of tools which combine microfluidic channels and electrodes in a single device, fabricated by hand and by a thermal draw process. Proof-of-concept results will be presented using flexible multimodal fibres to monitor transient neurochemical changes in an in vivo mouse model.3 The versatility and potential for multifunctional fibre sensors is immense, particularly for neural tissue monitoring. References: 1. Yu, P. et al. (2020). Angewandte Chemie, 132 (50), 22841. 2. Park, S. et al. (2017). Nature neuroscience, 20(4), 612. 3. Booth, M. A. et al. (2021). Analytical chemistry, 93(17), 6646.

NOVEL FRONTIERS IN FAST VOLTAMMETRY: NEW ANALYTES AND MODELS

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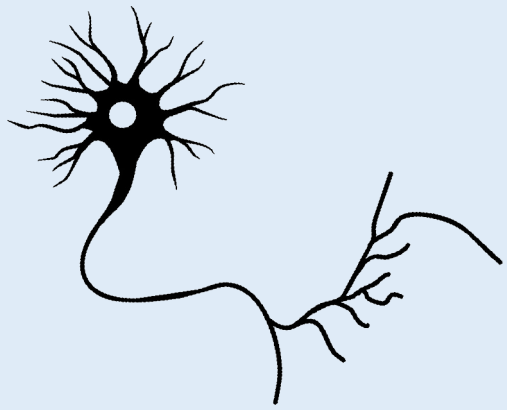
Keywords : Serotonin, histamine, FSCV, FSCAV, basal, microfluidics

Defining the chemistry of neurotransmission at high time resolution is important for understanding the physiology and pathology of the brain. Voltammetric methods at carbon fiber microelectrodes provide selective measurements of neurotransmitters *in vivo* with excellent temporospectral resolution. Fast scan cyclic voltammetry (FSCV) has traditionally been used to measure electrically or optically evoked dopamine *in vivo* and in tissue slice preparations, shedding a wealth of light onto dopamine's roles in the brain. In this talk, I will present novel frontiers in fast voltammetry by highlighting work our group has done in expanding the scope and capabilities of the technique. First, I will present our work expanding FSCV to serotonin and histamine in inflammation models. These models include measuring serotonin and histamine in the brain during acute and chronic inflammation, establishing the first measurements of serotonin and histamine from hair and skin cells and developing a novel microfluidic device with integrated carbon electrodes for serotonin and histamine detection from stem cell derived organoids. Second, I will present fast scan-controlled adsorption voltammetry (FSCAV), a method that reports minute to minute ambient levels of serotonin and histamine. Here, various novel experimental, analysis and physiological of FSCAV findings will be presented. In sum, the power and versatility of fast voltammetry for neuroscience research will be showcased,



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P07 DECRO SYSTEM FOR ENHANCED CIRCADIAN RHYTHM PHENOTYPING IN RATS Timothé Flénet, Agathe Cambier, Stéphane Baudet, Raafat Fares

P08 OPTIMIZING THE FABRICATION OF CARBON-FIBER MICROBIOSENSORS FOR SIMULTANEOUS DETECTION OF GLUCOSE AND DOPAMINE IN BRAIN TISSUE Alexandra G. Forderhase, Jack S. Twiddy, Lailah A. Ligons, Emilie M. Norwood, Gregory S. McCarty, Leslie A. Sombers

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P10 IMPLICATION OF POLYUNSATURATED FATTY ACID (PUFA) BIOSTATUS IN DOPAMINE TRANSMISSION-RELATED DEFICITS IN EXECUTIVE FUNCTIONS Lola Hardt, Florian Hontarrede, Julien Catanese, Anna Petitbon, Roman Walle, Pierre Trifilieff

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P12 SELF-REFERENCING APTAMER-FUNCTIONALIZED NANOPIPETTES Annina Stuber, Julian Hengsteler, Fiorella di Santo, Anna Burdina, Nako Nakatsuka

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P19 3D PRINTED MICROFLUIDIC DEVICES USED WITH DEXAMETHASONE-ENHANCED CONTINUOUS ONLINE MICRODIALYSIS Andrea Jaquins-Gerstl, Adrian C. Michael

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P22 ON-LINE AND IN VIVO ELECTROCHEMICAL ANALYSIS BASED ON METAL?ORGANIC FRAMEWORKS NANOZYME Yuqing Lin, Guoyuan Ren, Jia Liu

P23 MODELLING OF ANTIDEPRESSANT EFFECTS ON THE SEROTONIN SYSTEM Sergio Mena, Colby E. Witt, Melinda Hersey, Jordan Holmes, Anna Marie Buchanan, Lauren E. Honan, Brenna Parke, Shane N. Berger, Lauren Batey and Parastoo Hashemi

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P25 OPTIMISING FAST SCAN CYCLIC VOLTAMMETRY ANALYSIS FOR THE DETECTION OF DOPAMINE IN THE ZEBRAFISH BRAIN Oluwapelumi Obasaju, Andrew Young and William HJ Norton

P26 IN VIVO CHARACTERIZATION OF DOPAMINE SIGNALS BETWEEN STRIATAL SUBREGIONS IN FOUR DIFFERENT BEHAVIORAL PARADIGMS Pascal Warnaar, Eugenia Z Poh, Wouter van Elzelingen, Jessica Goedhoop, Ingo Willuhn

P27 ELECTROCHEMICAL DETECTION OF BEHAVIORALLY-EVOKED DOPAMINE RELEASE BY SUGAR FEEDING IN ADULT DROSOPHILA MUSHROOM BODY Mimi Shin and Jill B. Venton

3. Electrochemistry, electrophysiology – in vitro

P28 CHANGES IN DOPAMINE DYNAMICS AFTER ABSTINENCE FROM CHRONIC ETHANOL EXPOSURE IN MICE DORSAL AND VENTRAL STRIATUM I. Pamela Alonso, Yolanda Mateo, David M. Lovinger

P29 IN VITRO CHARACTERIZATION OF AN ELECTROCHEMICAL TECHNIQUE TO DETECT AMBIENT BRAIN HISTAMINE Lauren Batey, Sergio Mena, Colby E. Witt, Marsilea Booth, Parastoo Hashemi

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P31 METHAMPHETAMINE AND FENTANYL CO- SELF-ADMINISTRATION MODIFIES FENTANYL TAKING AND EXACERBATES MESOLIMBIC DOPAMINE DEFICITS Monica Dawes, Katherine Holleran, Sara Jones

P32 EXPLORING HOW PRESYNAPTIC CHOLINERGIC INPUT MODULATES DOPAMINE RELEASE IN MICE AND DROSOPHILA MELANOGASTER Lucille Duquenoy, Yan-Feng Zhang, Scott Waddell, Stephanie J. Cragg

P33 LONG-LASTING ALTERATIONS IN AXONAL DOPAMINE RELEASE REGULATION & UPSTREAM TRANSCRIPTION INDUCED BY ETHANOL DRINKING IN MACAQUES Zahra Z. Farahbakhsh, Katherine M. Holleran, Steve C. Fordahl, Verginia Cuzon Carlson, Kathleen A. Grant, Sara R. Jones, Cody A. Siciliano

P34 REDUCED DOPAMINE TERMINAL FUNCTION IN THE NUCLEUS ACCUMBENS FOLLOWING HEROIN SELF-ADMINISTRATION Brianna George, Monica Dawes, Gracie Peck, Sara R. Jones

P35 REAL TIME MEASUREMENTS OF DOPAMINE AND GLUTAMATE IN RAT STRIATUM USING FAST-SCAN CYCLIC VOLTAMMETRY Laney C. Kimble, Jack Twiddy, Jenna M. Berger, Jovica Todorov, Alexandra G. Forderhase, Gregory S. McCarty, John Meitzen, Leslie A. Sombers

P36 LIGHT STIMULATION REDUCTION IN NEURONAL ACTIVITY IS DEPENDENT ON THE TEMPORAL PATTERN AND LIGHT POWER USED Anistasha Lightning, Corinne Beurrier, Marie Bourzeix, Nicola Kuczewski

P37 INVESTIGATING THE REGULATION OF STRIATAL DOPAMINE RELEASE BY STRIATAL NOREPINEPHRINE AT ADRENERGIC RECEPTORS Jessica A. Livesey, Jeffrey Stedehouder, Jiesi Feng, Yulong Li, Mark E. Walton, Stephanie J. Cragg

P38 GESTATIONAL ETHANOL EXPOSURE INDUCES SEX-SPECIFIC IMBALANCES IN STRIATAL ACETYLCHOLINE AND DOPAMINE DYNAMICS Yolanda Mateo, Sebastiano Bariselli, Noa Reuveni and David M Lovinger

P39 CONVERGENT REGULATION OF PRESYNAPTIC SHORT-TERM PLASTICITY IN STRIATAL DOPAMINE RELEASE BY DOPAMINE TRANSPORTER & GABA RECEPTORS Bethan M. O'Connor, Emanuel F. Lopes, Katherine R. Brimblecombe, and Stephanie J. Cragg

P40 CHARACTERIZING SEROTONIN SIGNALING IN HUMAN EPIDERMAL CELLS WITH FAST SCAN CYCLIC VOLTAMMETRY Brenna Parke, Julia Agramunt, Sergio Mena, Melissa Hexter, Andrei S. Koslov, Claire Higgins, Parastoo Hashemi

P41 SYNAPTOGYRIN-3 PREVENTS COCAINE-INDUCED BEHAVIORAL AND DOPAMINERGIC ADAPTATIONS Gracie Peck, Katherine Holleran, Jason Locke, Paige Estave, Brian McCool, Sara Jones

P42 ATTENUATION OF STIMULATED ACCUMBAL DOPAMINE RELELASE BY NMDA IS MEDIATED THROUGH METABOTROPIC GLUTAMATE RECEPTORS Felicity Spencer, Maria Glodkowska, Anna Sebold, Ersin Yavas, Andrew Young

4. Fluorescence based-imaging

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SILICON NEUROPROBES ARE ASSOCIATED WITH REDUCED BRAIN TISSUE INJURY COMPARED TO MICRODIALYSIS PROBES

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Keywords: Inflammation, immunocytochemistry, astrocytes, microglia, biosensors

Implantable probes enable direct and indirect neurochemical monitoring *in vivo*. We have developed small Si neuroprobes for multiplexed neurotransmitter detection *in vivo.* Microfabricated neuroprobes have biosensors on their tips composed of field-effect transistors (FETs for signal transduction) coupled with aptamers (oligonucleotide receptors for target recognition). When neurotransmitters (or other targets) bind to aptamers, the negatively charged oligonucleotide backbones undergo conformation changes that gate FET transconductance in a target-concentration-dependent manner. Implanted devices induce brain injury and inflammation at implantation sites that can interfere with measurements, particularly when recording over longer time periods (days to weeks to months). In an initial study, we investigated brain inflammatory responses of similarly sized Si neuroprobes (150 mm x 150 mm) and microdialysis probes (240 mm diameter). Astrocytes and microglial cells are key mediators of inflammatory responses to implanted devices. We evaluated two markers–GFAP, the key cytoskeletal astrocyte intermediate filament protein, and CD11b, a marker for activated microglia. Probes were stereotaxically implanted into the hippocampus for acute (1 day) or subchronic (1 week) durations. Brains were perfused and horizontally sectioned at 40 µm. Anti-GFAP and anti-CD11b antibodies primary antibodies were visualized with secondary fluorescent antibodies and confocal microscopy. We found that the Si neuroprobes produced less pronounced astrocytic and microglial responses at the implantation site and in the surrounding tissue compared to microdialysis probes. Future experiments are focused on additional time points, blood vessel and other markers, smaller Si probes (50 mm x 50 mm), and neuroprobes fabricated on soft substrates (polyimide, polyethylene terephthalate) to determine how neuroprobe size and stiffness influence time-dependent inflammatory processes and how these compare with other common implanted devices.

HISTONE ACETYLTRANSFERASE KAT2A IS A CRITICAL EPIGENETIC REGULATOR OF COCAINE RESPONSES IN THE NUCLEUS ACCUMBENS

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Keywords: substance use disorder, cocaine, epigenetics, optical imaging, nucleus accumbens

Substance use disorder is characterized by cycles of drug-use, abstinence, drug-seeking, and relapse. The neural basis of these long-lasting behaviors has been linked to neural circuit function through changes in neurotransmission and receptor-based changes across the reward circuitry of the brain. The nucleus accumbens (NAc) is critical in the integration of rewarding and aversive information and is at the center of the neural dysfunction associated with drug addiction. At the center of this process is the ability of drug exposure to alter the inducibility of many genes in response to future drug-associated stimuli. To understand how drugs of abuse, such as cocaine, generate long-lasting behavioral changes, it is critical to link between neuronal activity and changes in gene expression. Our goal is to define how information about drugs is represented in the activity of specific neurons in the NAc, and how specific epigenetic signatures within these neurons is linked to drug seeking behavior. We identified temporally specific changes in Histone H3 post-translational modifications and identify a key regulator in these changes – KAT2A. Here, we utilize cocaine self-administration and *ex vivo* calcium imaging to identify the function of KAT2A in D1 medium spiny neurons (MSNs) in cocaine-seeking behavior. We find that loss of KAT2A function in D1-MSNs heightens cell excitability and alters sensitivity and motivation for cocaine. Moreover, we generate a cocaine self-administration activity profile of D1-MSNs that is subsequently altered by alterations in KAT2A function. The results of these studies contribute evidence for persistent cocaine-induced epigenetic adaptations and are the first step in generating a mechanistic link between epigenetic adaptations and changes in neuronal firing. In addition, we provide data linking these changes in epigenetic state to cocaine-seeking behavior.

THE EFFECT OF NANOSTRUCTURATION OF SEMI-CONDUCTOR OR POLYMER MATERIALS IN NEURAL CELL CULTURES: IMPLICATIONS FOR NEURAL IMPLANT DESIGN

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Keywords : nanowires, neurons, implant, electrophysiology, brain

Nanowires can be used in a broad range of bio-applications among which are neural implants for brain computer interface or neuroprostheses. We have shown that neurons from the Central Nervous System thrive when cultured on vertical arrays of semi-conductor nanowires (NWs), whereas the growth of glial cells on such arrays is limited compared to when cultured on flat substrates. However, semi-conductor nanowires present challenges in terms of integration in neural implants, such as their integration in a flexible substrate and their resistance to corrosion. We have analyzed the interaction of neuronal cells with NWs made from insulator polymers that are usually used for neural implants. For this purpose, we performed retinal and cortical cell cultures on SU8 and parylene-C polymer NWs. Four µm long SU-8 NWs positively influenced cell adhesion and neurite network formation compared to 1 µm long SU-8 NWs and flat SU-8 substrates. However, flat parylene-C was found to be the best polymer. Although we anticipate that parylene-C NWs might improve cell behavior, it has not yet been possible to obtain parylene-C NWs longer than 2µm. Taken together, these results suggest that arrays of nanowires are promising nanomaterials for designing neural interfaces and that the type of material and shape/dimensions of such nanomaterials play an important role.

A GENETICALLY ENCODED SENSOR FOR IN VIVO IMAGING OF OREXIN NEUROPEPTIDES

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Keywords : Genetically encoded Sensor, Orexins/Hypocretins, GPCRs, Fluorescence, neural activity

Orexins (also called hypocretins) are hypothalamic neuropeptides that carry out essential functions in the central nervous system; however, little is known about their release and range of action in vivo owing to the limited resolution of current detection technologies. Here we developed a genetically encoded orexin sensor (OxLight1) based on the engineering of circularly permutated green fluorescent protein into the human type-2 orexin receptor. In mice OxLight1 detects optogenetically evoked release of endogenous orexins in vivo with high sensitivity. Photometry recordings of OxLight1 in mice show rapid orexin release associated with spontaneous running behavior, acute stress and sleep-to-wake transitions in different brain areas. Moreover, two-photon imaging of OxLight1 reveals orexin release in layer 2/3 of the mouse somatosensory cortex during emergence from anesthesia. Thus, OxLight1 enables sensitive and direct optical detection of orexin neuropeptides with high spatiotemporal resolution in living animals.

WORKING TOWARDS SIMULTANEOUS MONITORING OF DOPAMINE RELEASE AND D1-NEURON ACTIVITY IN THE NUCLEUS ACCUMBENS OF FREELY MOVING ANIMALS

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Keywords: dopamine; fiber photometry; basal-ganglia direct pathway; striatum; calcium imaging

Medium-spiny projection neurons make up about 95% of the neurons in the striatum and form the starting point of the two output pathways of the basal ganglia. These pathways are characterized by the relatively exclusive expression of D1-type and D2-type dopamine receptors that bias post-synaptic activation towards excitation and inhibition, respectively. The pathways are thought to play distinguishable roles in behavior; the D1 pathway is implied in behavioral activation, while the D2 pathway is linked to behavioral inhibition. Even though dysfunction of these two pathways is implied in a multitude of psychiatric disorders, the modulating role of dopamine release on either D1 or D2 neurons has not been systematically studied in freely moving animals. We use fiber photometry to investigate both the endogenous release of dopamine and associated activity of D1 neurons in the nucleus accumbens core (NAcc) of a D1-Cre transgenic rat line. We inject D1-Cre rats intra-cranially with a combination of viruses resulting in the Cre-specific expression of GCaMP6f and Cre-independent expression of RdLight. This aims at the simultaneous measurement of dopamine release and post-synaptic D1 neuronal activation, while rats are exposed to a series of behavioral paradigms involving both appetitive and aversive stimuli. Preliminary results show that D1 neurons in the NAcc respond to both appetitive and aversive stimuli. We currently conduct follow-up experiments that include simultaneous sensing of dopamine release and calcium influx. Based on our recently published work we expect opposing effects of stimulus valence on dopamine concentration in the NAcc: using fast-scan cyclic voltammetry (FSCV), we detected an increase in response to appetitive stimuli and a decrease to aversive stimuli. If replicated, these results would point at an unexpected schism of dopamine release and post -synaptic D1-neuron activity that we will then examine more closely trial by trial. Additional experiments are currently performed and analyzed.

MICROELECTRODE-BASED BIOSENSORS FOR REAL-TIME DETECTION AND MONITORING OF LACTATE IN VIVO IN THE BRAIN

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Keywords: Electrochemical biosensor; Lactate; In vivo monitoring.

The role played by lactate in brain bioenergetics has changed considerably during the past decades. Lactate is a critical glycolytic metabolite trafficked between astrocytes and neurons, also acting as an intercellular signalling molecule. Hence, it is essential to monitor extracellular lactate concentrations to understand its role in brain metabolism. Microbiosensors coupled with fast electrochemical techniques have been used as an attractive analytical tool for monitoring the concentration dynamics of nonelectroactive neurotransmitters and metabolic substrates in the brain extracellular space with high spatial and temporal resolution. Additionally, measurements can be performed with high sensitivity, selectivity, and minimal tissue damage. In the present work, microelectrode-based biosensors have been designed and developed using ceramic-based microelectrode arrays (MEA) and platinum modified carbon fiber microelectrodes (CFM/Pt) as sensing platforms for the detection and monitoring of lactate. Lactate oxidase (LOx) was immobilized on the electrode surface in the presence of bovine serum albumin (BSA) by using glutaraldehyde as cross-linking agent. To extend the microbiosensor linear range for the substrate quantification, the microelectrodes were further coated with an exclusion layer of polyurethane (PU). The enzyme loading, BSA concentration, enzyme immobilization and PU layers were optimized to enhance microelectrode performance. The morphological characteristics and electroanalytical performance of the microbiosensors were assessed, by scanning electron microscopy and electrochemical techniques. Before insertion into the rat brain the microbiosensors were coated with exclusion layers of Nafion® and/or meta-phenylenediamine (m-PD) to minimize the interference of undesired compounds such as ascorbate. The microbiosensors were successfully used to detect and monitor lactate with high spatial and temporal resolution in vivo in response to stimulated changes in lactate concentration. (Funding: POCI-01-0145-FEDER-028261).

DECRO SYSTEM FOR ENHANCED CIRCADIAN RHYTHM PHENOTYPING IN RATS

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Keywords : Circadian rhythm, activity, cardiorespiratory, jacketed telemetry, non-invasive

Circadian phenotyping in rodents is usually established by actogram plotting using a variety of non-invasive setups (software and hardware). In neuroscience research field, monitoring of circadian rhythm can be useful and informative and, in some circumstances, combining cardiorespiratory parameters to this actogram can become an added value. However, this combination implies that the setups become either invasive (telemetry implant) or restraining (plethysmography). DECRO™ system has been developed to refine the implementation of this combination in a non-invasive and a non-restraining manner.

Data from 8 Sprague Dawley rats (229-256 g), used in a safety pharmacology study, were reanalysed to assess the ability of DECRO system to detect circadian rhythms for several vital functions. Rats were pair-housed with a 12-hour Light/Dark cycle were divided in two groups (Vehicle and Baclofen 15 mg/kg *P.O.*). Heart Rate (HR), Respiratory Rate (RR) and Activity Level (AL) were recorded over 24 hours using a DECRO Bluetooth-based, external jacketed telemetry device. Data were averaged for the 12 -hour light/dark periods.

In the vehicle group, the AL during the dark period (active period for rats) was found significantly higher ($p<0.05$) than during the light period (+22 mg / +133%). HR and RR followed the same trend (+33 bpm / +8%; +9 bpm / +6%; respectively).

In the baclofen group, the signature of the circadian rhythm was altered by the pharmacological effect of the compound (activity depressant). Indeed, although the AL increased during the dark period, its magnitude was lower than in the control group.

We provide evidence that the DECRO system can detect activity level and cardiorespiratory changes whether being related to circadian rhythm or to a test compound. Moreover, this approach is in line with the 3R rule by its non-invasiveness and can be considered as an added value for neuroscientists.

OPTIMIZING THE FABRICATION OF CARBON-FIBER MICROBIOSENSORS FOR SIMULTANEOUS DETECTION OF GLUCOSE AND DOPAMINE IN BRAIN TISSUE

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Keywords: enzyme, voltammetry, real-time, electrodeposition, characterization

Neuronal communication is an energetically demanding process; while the brain comprises only 2% of the body's total mass, it consumes 20% of the body's glucose. There is substantial evidence implicating a dysregulation in both brain energy metabolism and dopaminergic function in several neurological disorders, including Alzheimer's disease and addiction. However, the precise relationship between dopamine signaling and glucose/lactate availability remains unclear, largely due to a critical lack of analytical tools capable of simultaneously monitoring these species at discrete locations in brain tissue. Carbon-fiber microelectrodes are commonly coupled with fast-scan cyclic voltammetry (FSCV) for the detection of rapid dopamine transients in situ. These electrodes can be modified with oxidase enzymes to create microbiosensors capable of simultaneously quantifying real-time fluctuations of non-electrochemically active substrates, such as glucose or lactate. Hydrogel entrapment of the oxidase enzyme within a chitosan matrix on the carbon surface provides for stable, sensitive, and selective detection of dopamine and the enzyme substrate using FSCV. The purpose of this study is to characterize the physical nature of the hydrogel, and its effects on the acquired electrochemical data. The chitosan hydrogel was deposited using linear sweep voltammetry, and membrane consistency and electrochemical performance were characterized to optimize the potential range and deposition rate. Electrochemical impedance spectroscopy was used to relate impedance and capacitance measurements to sensor performance before and after electrodeposition, as well as after electrochemical conditioning. Finally, these factors were directly related to the voltammetric performance to provide a handle to tune sensitivity and selectivity to these analytes. Overall, these experiments are important because they provide an improved understanding of the hydrogel matrix that is integral to microbiosensor function, thus advancing this much-needed technology for monitoring real-time neurochemical kinetics.

PORTABLE MICROFLUIDIC BIOSENSING SYSTEM FOR HIGH-TEMPORAL RESOLUTION ANALYSIS OF BRAIN MICRODIALYSATE IN REAL TIME

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Keywords: Biosensors, neurochemistry, microfluidics, microdialysis

In vivo neurochemical monitoring can provide clinicians with vital information about brain physiology and pathology and can be used to direct patient care. *In vivo* neurochemical monitoring has been successfully achieved using continuous online microdialysis. In crowded clinical environments, however, such an approach typically involves long connection tubing leading to a delay in detecting dynamic changes. A high-temporal resolution sensing system that can be placed closer to the patient would allow real-time monitoring of tissue health, significantly aiding clinical decision-making and patient outcome.
We have developed a monitoring platform comprising a portable analysis system and a programmable microfluidic workstation.[1] The analysis system uses sensors to quantify key markers of ischaemia in real time. Microdialysis is used to sample the tissue and the resulting dialysate is monitored for changes in levels of metabolites and ions using microelectrode-based sensors.[2,3] The sensors are housed within an optimised low-volume 3D-printed microfluidic chip[4] to continuously measure changes in these key analytes in real time. The portable platform also incorporates wireless battery-powered control electronics, which link to a tablet via Bluetooth for real-time visualisation of changes as they occur.

Proof-of-concept results will be presented showing these new technologies applied in a porcine model to monitor vulnerable brain tissue during cardiac arrest and resuscitation, demonstrating the potential of this methodology for real-time clinical monitoring.

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IMPLICATION OF POLYUNSATURATED FATTY ACID (PUFA) BIOSTATUS IN DOPAMINE TRANSMISSION-RELATED DEFICITS IN EXECUTIVE FUNCTIONS

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Keywords : Executive functions, Polyunsaturated Fatty Acids, Dopamine, D-light,

Deficits in executive functions is a characteristic symptom found across various psychiatric diseases such as schizophrenia, major depression or bipolar disorder. These deficits have been related to a dysfunction of the prefrontal cortex (PFC), and notably an alteration of mesocorticolimbic dopamine (DA) transmission. However, the underlying pathophysiological mechanisms remain unclear. Many clinical studies have described a decrease in n-3 Polyunsaturated Fatty acids (PUFAs) in subsets of patients suffering from these psychiatric disorders. Interestingly, preclinical findings from others and our lab suggest that dopamine transmission is particularly sensitive to PUFA biostatus. We therefore hypothesized that developmental n3 PUFA deficiency could lead to impaired executive functions at adulthood through a direct effect on cortical dopamine transmission. We combined genetically encoded biosensor d-light coupled to fibre photometry as well as anatomical and biochemical analyses to assess the integrity of DA transmission in the medial PFC of n-3 PUFA deficient mice. Executive functions were tested through operant conditioning based tasks probing for the ability of animals to adapt their behaviour in the face of changes in outcome value and action-outcome contingency. Finally, to assess whether n3 PUFA deficiency in DA neurons is sufficient to induce executive function impairments, we restored PUFA levels selectively in DA neurons through a transgenic mouse model (iFAT1). These findings further suggest that dopamine transmission is particularly vulnerable to PUFA biostatus and support an implication of lipid metabolism in the aetiology of specific psychiatric symptoms such as deficits in executive functions.

NANOSCALE APTAMER-MODIFIED BIOSENSORS MONITOR DOPAMINE AND SEROTONIN EX VIVO

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Keywords: DNA, nanobiosensor, nanopore, optoelectronic sensing, multiplex

Measuring specific chemical interactions at the spatiotemporal resolution that approaches biologically meaningful dimensions and timescales is critical for understanding neuronal communication. While advanced methods to record electrical signaling from neurons are prevalent, tools to monitor neurochemical signaling have been limited. We have tackled this challenge by coupling the inherent selectivity of DNA-based recognition elements termed aptamers, with nanoscale pipettes with openings of ca. 10 nm. Aptamers are systematically designed oligonucleotide receptors that exhibit highly specific and selective recognition of targets. Aptamers that recognize small-molecule neurotransmitters, including serotonin and dopamine, have recently been isolated. Upon reversible target binding, aptamers undergo a rearrangement of the negatively charged backbone, and these dynamic structural changes can be transduced as measurable changes in current through the nanoscale orifice of the sensors. Nanoscale confinement of the sensor surface reduces biofouling for long-term recordings in complex environments, overcoming a critical bottleneck for clinical biosensors. We have demonstrated the capacity to detect physiologically relevant differences in neurotransmitter amounts released by live neurons in complex media with unprecedented sensitivity. We are now expanding our technology to measure chemical flux of dopamine in localized brain regions in situ (e.g., striatum, cortex) upon electrical and chemical stimulation of neurotransmitter release. In parallel, we are coupling electronic sensing with optical sensors (genetically encoded dopamine sensors, dLight1) to expand the detection window and enable simultaneous readout of events at nanoscale and cellular resolutions. Further, we aim to target diverse targets beyond serotonin and dopamine through this generalizable method and to multiplex sensors for multi-chemical recordings.

SELF-REFERENCING APTAMER-FUNCTIONALIZED NANOPIPETTES

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Keywords: Dopamine, Serotonin, Nanopore, Multiplex, Biosensing

Quantitative and real-time measurement of neurotransmitters in complex environments is crucial to understand the basics of neurological function and neurodegenerative diseases. To measure chemical signaling in the brain dynamically, aptamer-functionalized nanopipettes have been developed and validated in complex media and in brain tissue. Aptamers are single-stranded DNA specifically designed to interact and bind with a target of interest (*e.g.,* dopamine, serotonin). Robust surface chemistry is used to couple DNA aptamers inside solid-state nanopores in the form of glass nanopipettes with ~10 nm orifices. Upon target-specific binding, the aptamers undergo a significant confirmational change, leading to a rearrangement of the highly negative DNA backbone, and enabling detection of a change in current. The nanoscale dimensions and high selectivity of these sensors enables unprecedented precision with minimal nonspecific interactions and binding compared to advanced state-of-the-art techniques such as microdialysis or fast scan cyclic voltammetry. Measurements of neurochemicals in complex environments with high amounts of interferents and constant ionic flux, necessitates differential recordings in localized regions to differentiate specific *vs.* nonspecific signals. To this end, we are developing novel platforms and optimized surface chemistry strategies to enable self-referencing of the specific aptamer sensor with a control sensor. We functionalize the reference sensor with a DNA sequence containing the same number and type of DNA bases as the specific sensing aptamer, but in a scrambled order, hindering target recognition. The reference sensor will observe the same environmental changes with the same chemical signature as the specific sensor, while negligibly responding to the neurotransmitter of interest upon exposure. Further, such multiplexing strategies will enable the fabrication of sensors that can monitor multiple different neurotransmitters simultaneously.

LUMATEPERONE INCREASES GLUTAMATE RELEASE IN THE RAT MEDIAL PREFRONTAL CORTEX, MEASURED WITH AMPEROMETRY AND ELECTROPHYSIOLOGY

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Keywords : Lumateperone, Antipsychotic drugs, Glutamate, Schizophrenia, Amperometry

Schizophrenia is a severe psychiatric disorder affecting approximately 20 million people globally. Lumateperone is a new antipsychotic drug that is a 5HT2A antagonist, a post-synaptic dopamine D2 antagonist, a presynaptic dopamine D2 partial agonist, a dopamine D1 modulator, a serotonin reuptake inhibitor and enhances phosphorylation of GluN2B receptors at tyrosine residue Y1472. Clinically, lumateperone effectively ameliorates the clusters of symptoms in adult patients with schizophrenia. Moreover, lumateperone has a safety profile similar to placebo for extrapyramidal symptoms, prolactin changes and cardio-metabolic effects. As cortical *N*-methyl-D-aspartate (NMDA) receptor hypofunction is hypothesized to be involved in the pathophysiology of schizophrenia, the aim of the current study was to investigate the effect of lumateperone on glutamatergic neurotransmission in the rat medial prefrontal cortex (mPFC).

We utilized enzyme-selective microelectrode arrays combined with amperometry to measure glutamate levels in anaesthetized rats. Moreover, by using electrophysiology *in vitro*, we investigated the effect of lumateperone on NMDA- and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-induced currents.

In the mPFC of anaesthetized rats, lumateperone caused a significant increase in glutamate release (0.38 µM increase from baseline) compared to vehicle. The increase of glutamate was rapid (within 20 minutes post systemic injection) with a sustained duration of ~100 min. Moreover, in brain slices including the mPFC, we found that lumateperone significantly increased both NMDA- and AMPA-induced currents, which could be prevented by a selective dopamine D1 antagonist SCH23390.

In summary, we show that lumateperone significantly increases glutamate release in the mPFC of anaesthetized rats, and facilitated NMDA and AMPA receptor-mediated currents in the mPFC, in a dopamine D1-dependent manner. The effect of lumateperone on glutamatergic neurotransmission may be of great importance for the improvement of depressive and cognitive symptoms in schizophrenia, a crucial cluster of symptoms that is currently difficult to treat.

EXPLORING INTERSTITIAL CALCIUM DYNAMICS USING NOVEL GENETICALLY ENCODED INDICATORS

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Keywords: Interstitial, fluorescence, calcium, *in-vivo*, biosensors

Genetically encoded indicators are invaluable tools for *in-vitro* and *in-vivo* calcium imaging. During the last decades, different structural designs allowed to shed light into the role of intracellular calcium dynamics in a wide range of biological processes. In this work, we designed and optimized a new genetically encoded single-fluorophore indicator combining the minimum calcium-binding domain from Troponin-C and the fluorescent protein mNeonGreen. Using an iterative approach between directed evolution and extensive biophysical characterization of the libraries, we were able to generate a large family of highly sensitive biosensors for a wide range of calcium concentrations.

In particular, we will present members of this new family of indicators that were specifically tuned to monitor calcium dynamics in the interstitial space, where the concentrations are expected to be four to five orders of magnitude higher than in the cytosol. Therefore, an interstitial biosensor is required to detect relatively small calcium transients on extremely high calcium backgrounds. After an extensive characterization in solution and mammalian cells, we confirmed the ability of our indicator to detect interstitial calcium changes in rat brain slices and zebrafish under physiological conditions.

PHOSPHODIESTERASE 2A : FUNCTIONAL ROLE IN THE STRIATUM AND POTENTIAL AS A NEW THERAPEUTIC TARGET IN PARKINSON'S DISEASE

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Keywords : Biosensor imaging; cAMP/PKA signalling; dopamine; striatum; Parkinson's disease

Aims – In Parkinson's disease (PD), the degeneration of dopaminergic neurones results in a deficit of dopamine. This situation is commonly treated pharmacologically, by the administration of L-DOPA, a precursor of dopamine. However, after about 10 years of treatment, 80% of patients develop L-DOPA-induced dyskinesia (LID). In the dopamine depleted striatum, D_1-type medium-sized spiny neurones (D1 MSN) become hyper-responsive to the stimulation of type 1 dopamine receptors. This hypersensitivity leads to an over-activation of the cAMP/PKA signalling pathway, resulting in the progressive development of LID. Our aim is to evaluate the potential of phosphodiesterase 2A (PDE2A), which degrades cAMP, to reduce D_1 MSN hypersensitivity associated with LID. Because of its low affinity for cAMP, the stimulation of PDE2A activity through the NO/cGMP pathway could reduce excessive cAMP levels while preserving proper responses.
Methods – Biosensor imaging reports the dynamics of cAMP/PKA signalling in MSNs in striatal brain slices from young mice, or adult mice in PD and dyskinetic situation.
Results – In PD and dyskinetic mouse model, D_1 MSN display a larger cAMP response to transient dopamine compared to normal mice. The larger cAMP response is similar to the response measured in immature brain. Interestingly, PDE2A activation by the NO/cGMP pathway efficiently reduces the amplitude of the dopamine response in PD and dyskinetic mouse model.
Conclusion – the stimulation of PDE2A activity moderates excessive cAMP levels in the response to dopamine in dyskinetic mice. These results highlight the therapeutic potential of PDE2A activation in the treatment of LID.

3-D SIMULATIONS USED TO INVESTIGATE ELECTROENZYMATIC GLUTAMATE SENSOR MINIATURIZATION FOR IMPROVED SENSITIVITY AND SPATIAL RESOLUTION

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Keywords: biosensors, electrochemistry, in vivo, electrochemistry, in vitro, simulations, glutamate

Highly selective, implantable electroenzymatic glutamate sensors with permselective polymer coatings and immobilized glutamate oxidase enzyme have been improved to near the theoretical limits with the aid of 1-dimensional models: measured sensitivities and response times *in vitro* for 6000 μm^2 sensing sites in a 4 ´ 4 microelectrode array have been reported at 320 nA μM^{-1} cm^{-2} and 80 ms. However, optimization of the size, number, and placement of sensing sites on an array remains to be accomplished and is particularly relevant for understanding (and important for improving) the spatial resolution of sensors *in vivo*. To investigate the effects of miniaturization and probe design on sensor performance as well as the spatial resolution of these sensors, our prior 1-D models of sensor performance were expanded to 3 dimensions to enable more informative simulations of sensor responses to various glutamate release scenarios *in vivo*.

Simulations show that miniaturization of electroenzymatic sensors is feasible and is necessary to enable detection of synaptic excitation from smaller populations of nearby synapses. For example, miniaturization of sensors from 25 µm to 5 µm in radius likely would enable detection of glutamate release from thousands (rather than hundreds of thousands) of synapses excited simultaneously for ~0.5 s. The method of enzyme deposition is also shown to affect sensor performance: microstamping of enzyme onto areas that extend slightly beyond (vs. tens of microns beyond) the edge of microelectrode sites can reduce the uncertainty in sensor response caused by expected variations in local biological regulatory processes. Microstamping in this way also halves the levels of H_2O_2 generated by the sensor, reducing the biological impact (although in all cases local glutamate concentrations are affected within ~20 µm). It is also determined that sensors can be situated much more closely (<5 µm) on sensor probes without risk of crosstalk between sensor sites. This new understanding of the spatial resolution and the benefits of miniaturization are expected to lead to improved sensor development and more accurate and informed interpretations of sensor data.

UNDERSTANDING THE ROLE OF MICROGLIA TO MODULATE RAPID ADENOSINE TRANSIENTS IN BRAIN

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Keywords : Adenosine, Fast scan cyclic voltammetry, Microglia

Adenosine plays an important role as a neuromodulator in the central nervous system (CNS). CNS not only produces adenosine but also is regulated by adenosine. Cells of the CNS, such as microglia, pericytes and neuronal cells can produce adenosine or their activity is regulated by adenosine. In the brain, microglia play countless number of supportive roles including sensing, intercellular communication, promotion of inflammation, degradation and repair. Microglia respond to neuronal activation by suppressing neuronal activity, and ablation of microglia amplifies and synchronizes the activity of neurons, leading to seizures. Recent study showed that mice treated with the CSF1R inhibitor PLX5622 chow for more than one week leads to a 99% microglia loss. In this study, we used fast scan cyclic voltammetry technique to study rapid adenosine transients in the caudate-putamen and hippocampus regions of the anesthetized mice. Transient adenosine measurements were carried out continuously for two hours in mice treated with PLX5622 and control chow. Our findings suggest that, both caudate putamen and hippocampus showed significant difference in the average event adenosine concentration and inter-event time but no change in the number of transients and cumulative adenosine. Overall, microglia play a small role on spontaneous adenosine after the treatment with PLX5622 diet.

A NEUROCHEMICAL STUDY OF ATYPICAL DAT INHIBITORS AS POTENTIAL THERAPEUTIC OPTIONS FOR PSYCHOSTIMULANT USE DISORDER

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Keywords: Dopamine, psychostimulant use disorder, DAT, FSCV, addiction

Psychostimulant use disorder (PSUD) is an increasingly prevalent condition with no FDA approved pharmacological treatments. Potential therapeutic options currently being explored include atypical dopamine (DA) uptake inhibitors (DUIs), which produce behavioral and neurochemical effects inconsistent with those elicited by typical abused psychostimulants. Modafinil (MOD), an agent approved for treatment of sleep disorders, is a low affinity DUI with a pharmacological profile different from cocaine. MOD has been suggested as a potential PSUD medication but with limited efficacy in selected addicted individuals. To improve efficacy, analogs of MOD with different pharmacological profiles have been synthesized. Similarly, rimcazole (RIM), a DUI and an antagonist of σ1 receptors, has shown potential efficacy in preclinical models of PSUD. Nucleus accumbens shell (NAS) DA transmission plays a role in PSUD, and here we employed fast scan cyclic voltammetry (FSCV) for investigation of evoked DA release/clearance in the NAS of Sprague Dawley rats. Pretreatments with the MOD-analogs, JJC8-088 (3-10 mg/kg, i.p.) and JJC8-091 (10-32 mg/kg, i.p.), produced divergent effects on NAS DA dynamics elicited by i.v. cocaine administration (0.1, 0.3, and 1.0 mg/kg). JJC8-088, a typical, cocaine-like DAT inhibitor, enhanced cocaine-induced increases in evoked DA release suggesting an additive effect of these compounds. In contrast, JJC8-091, an atypical DAT inhibitor that elicits limited, if any, cocaine-like stimulant effects, produced mild reductions in cocaine-induced stimulation of evoked DA release. Both JJC8-088 and JJC8-091 produced modest slowing of DA clearance both alone and in combination with cocaine. RIM (10 mg/kg, i.p.) also reduced cocaine-induced stimulation of evoked DA release, and even though it significantly attenuates the rate of DA clearance when injected alone, it does not appear to have any added effects on DA clearance when administered in combination with cocaine (0.1, 0.3, and 1.0 mg/kg). Our findings suggest that both JJC8-091 and RIM blunt cocaine neurochemical effects, indicating their potential efficacy for the treatment of PSUD.

3D PRINTED MICROFLUIDIC DEVICES USED WITH DEXAMETHASONE-ENHANCED CONTINUOUS ONLINE MICRODIALYSIS

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Keywords : Dexamethasone-enhanced microdialysis, 3D printed platform

There are many challenges associated with making microdialysis measurements in the clinical environment. Translating technological advances in online analysis from the laboratory to the clinical setting can be difficult. The Boutelle group has developed continuous online microdialysis (coMD) that uses biosensors to rapidly monitor glucose, lactate, and glutamate in the dialysate sample stream (1-4). They have developed a simple, robust, and user-friendly automated platform. Our goal is to explore highly innovate technologies to enhance coMD. We wish to advance from diagnosis to novel therapy to prevent secondary brain injury. Herein, we describe an improved dexamethasone-enhanced continuous online microdialysis system that is automated, robust, and fast enough to measure dynamic chemical changes in the brain. At present, microdialysis data are not shared with physicians who are barred from using such data to inform their medical decisions. In order for this to happen it is critical that measurements are made accurately, quick and utilize a simple platform.

Our study (1) demonstrates a 3D printed microfluidic platform design which allows for detection of the glucose, this platform incorporates (2) needle-style microelectrodes, 3D printed electrode holders and chips and allows for these sensors to be calibrated automatically. We have also (3) characterized these sensors by examining sentinel sensors, looked at the position of the sensors and tested their longevity and sensitivity. Lastly, we performed (4) in vivo data in which retrodialysis of glucose was performed on a rat over 2 days. As before, we have used dexamethasone (DEX), a powerful anti-inflammatory agent as a simple and effective mitigation strategy. From this, we conclude that innovate technologies can be used to enhance coMD. We have addressed an unmet need in translation of bench side methodology to those of clinical environments. This system provides real-time data which would allow doctors to respond to physiological changes quickly and efficiently.

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INTERFACING APTAMERS WITH ELECTROCHEMICAL SYSTEMS: SELECTIVELY PROBING NEUROCHEMICALS IN LIVING ANIMALS

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Keywords: In vivo analysis, electrochemistry, aptamers, neurochemicals

Probing the chemical and spatiotemporal information of neurochemicals is essential for understanding the molecular mechanism of brain function and pathology. In vivo electroanalytical systems using carbon fiber microelectrode (CFE) features excellent temporal/spatial resolution, while is fundamentally limited by the lack of interfacial selectivity. Aptamers (apt) are excellent recognition element, integrating them with in vivo compatible CFE offers a plausible means to endow the electrochemical sensors with greatly improved selectivity, however, this is significantly challenged in terms of coupling chemistry, stability, and versatility. In this talk, I will be presenting a recently developed interfacial functionalization strategy that allows the assembly of aptamers on CFE, generating aptCFE sensors that achieve selective recognition of neurotransmitter in living animals. The sensor was created through the assembly of aptamer cholesterol amphiphiles (aptCAs) onto the alkyl chain-functionalized CFE (Scheme 1). We show that the noncovalent cholesterol-alkyl chain interactions are capable of effectively immobilizing aptamers onto CFE surface. Moreover, the strategy is generalizable, facile, and applicable of synergizing the merits of microelectrode and aptamers, allowing the generation of a highly selective bioelectronic system for probing neurochemical dynamics in living systems. To our knowledge, this is the first demonstration of using noncovalent cholesterol-based anchoring of aptamers to carbon-based electrodes, opening up a vast array of new opportunities for designing in vivo sensors to exploring brain chemistry.

MANUFACTURING ENZYME MODIFIED CARBON-FIBER ELECTRODES FOR REAL-TIME MONITORING OF ELECTROACTIVE AND NON-ELECTROACTIVE SPECIES

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Keywords: Glucose, Dopamine, FSCV, Biosensor, Multianalyte

Translating new technology from the laboratory to the marketplace is difficult. We will discuss these challenges for 7 µm Dopamine (DA)/Glucose enzyme-modified electrodes developed at North Carolina State University by Dr. Leslie Sombers' group and commercialized by Pinnacle Technology. A successful bench-to-marketplace translation requires development of a robust manufacturing protocol that can deliver a high yield of conforming product (> 95% meeting specification goals), a reliable protocol for shipping and storage, calibration metrics, and assurances that the product will arrive safely, on time, and perform as advertised. The sensor specification goals were DA sensitivity > 5.0 nA/µM, glucose sensitivity > 10 nA/mM, and shelf life of 2 weeks. We will discuss the detailed development steps required to achieve these goals. This includes enzyme sourcing and validation procedures, hydrogel deposition and electrochemical conditioning optimization, storage media optimization, shelf-life testing, and manufacturing and shipping procedure development. At the conclusion of this project, the completed sensors exhibit a linear response to glucose up to 2 mM consistent across a wide O_2 concentration range (25-200 µM), DA sensitivity 34 +/- 12 nA/µM, glucose sensitivity 16 +/- 4 nA/mM, and a shelf life of 4 weeks.

Research reported in this abstract was supported by National Institute of Mental Health of the National Institutes of Health under award number R43MH118770.

ON-LINE AND IN VIVO ELECTROCHEMICAL ANALYSIS BASED ON METAL−ORGANIC FRAMEWORKS NANOZYME

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Keywords: In vivo; On-line electrochemical analysis; metal−organic frameworks nanozyme; Brain Analytical Chemistry

Continuous recording of the dynamic changes of a variety of neurochemicals in the body is very important to understand the molecular basis of brain function. The in vivo online analysis system combined with microdialysis technology has successfully detected the dynamic changes of a variety of neurochemicals in the brain and has become the commonly used means to continuously obtain the changes of chemical information in the brain in vivo. Nanozymes simulating natural enzyme activity are widely used to replace natural enzymes in analytical chemistry methods because they can work normally in non-physiological environments. Due to its unique structure and high porosity, metal−organic frameworks (MOFs) can act not only as nanozyme materials but also as carriers to encapsulate natural enzymes and thus have received extensive attention in recent years. Through the design and regulation of MOF-based nanozyme materials, many sensing methods with high stability and selectivity can be developed. By loading these sensing methods into the in vivo online analysis system, continuous analysis methods with better detection stability and selectivity can be obtained. Research examples are as follows:

1) We presented structure defects of MOFs as a tuning strategy to regulate the catalytic efficiency of artificial nanozymes and investigated the roles of defects on the catalytic activity of oxidase-like MOFs. This nanozyme-based microreactor can completely remove ascorbic acid, dopamine, and 3,4-dihydroxyphenylacetic acid which are the main interference toward uric acid (UA) electrochemical measurement, and the ZIF-L-Co-10 mg Cys-based OECS system is capable of continuously capturing UA change in rat brain following ischemia−reperfusion injury.

2) The “raisin pudding”-type ZIF-67/$Cu_{0.76}Co_{2.24}O_4$ nanospheres with multiple enzymelike activities were obtained. Based on its laccase-like activity, an online electrochemical system for continuous monitoring of 3,4-dihydroxyphenylacetic acid with good linearity in the range of 0.5−20 μ M and a detection limit of 0.15 μ M was established. Furthermore, the alteration of DOPAC in the brain microdialysate before and after ischemia of the rats’ brain was also successfully recorded.

MODELLING OF ANTIDEPRESSANT EFFECTS ON THE SEROTONIN SYSTEM

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Keywords : depression, serotonin, histamine, antidepressants, fast voltammetry.

Depression is one of the leading causes of disability worldwide, and its prevalence is expected to increase. Clinically, depressive patients have been treated over the years with a type of pharmaceutical agents called antidepressants, but their efficacy is low and usually a trial-and-error process is required to optimize the response of the patient to the treatment. The most prescribed antidepressants nowadays are selective serotonin reuptake inhibitors, and their main function is to bind to serotonin transporters and block the reuptake of serotonin back into neuronal terminals. There are, however, a range of other pharmacological compounds prescribed for the treatment of depression with different mechanisms of action, such as reboxetine and the newly FDA-approved ketamine. Despite showing some clinical efficacy, the complete physiological effect of these drugs on the serotonin system is still not clearly known. In this work, we show that acute administrations of these compounds with equivalent doses have an analogous effect on the levels of extracellular hippocampal serotonin in mice. Following, we mathematically model electrically evoked serotonin reuptake kinetics under acute antidepressant administration to study the different effects of these drugs on the serotonin neural release and reuptake mechanisms. Finally, we show the main pathways these drugs influence the extracellular levels of serotonin. These findings suggest that serotonin could be used as a unique biomarker for the diagnosis of depression and the effectiveness of new proposed treatments.

RAPID PULSE VOLTAMMETRY (RPV) COUPLED WITH PARTIAL LEAST SQUARES REGRESSION (PLSR) FOR MULTIPLEXED DETECTION OF SEROTONIN AND DOPAMINE

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Keywords : electrochemistry, *in vivo*, machine learning

A number of voltammetry methods have been developed to monitor brain extracellular dopamine and serotonin levels. No voltammetric techniques are currently available to monitor both neurotransmitters simultaneously across timescales (phasic and tonic), despite the fact that they play integrated roles in modulating behavior. Here, we report on rapid pulse voltammetry coupled with partial least squares regression (RPV-PLSR), an approach adapted from multi-electrode systems (*i.e.,* electronic tongues), which have been used to identify multiple components in complex environments. Using an intelligently designed pulse strategy, we constructed RPV waveforms by exploiting small differences in analyte redox profiles. In addition to faradaic currents, capacitive currents were important factors in analyte identification, precluding background subtraction and allowing both stimulated and basal measurements to be made in a single technique. Compared to fast-scan cyclic voltammetry-principal components regression (FSCV-PCR), RPV-PLSR better quantified and identified injected boluses of serotonin and dopamine *in vitro*. In a preliminary *in vivo* experiment, RPV-PLSR simultaneously differentiated and quantified basal and stimulated dopamine and serotonin associated with striatal recording electrode position, optical stimulation frequency, and pharmacology. We demonstrate that the RPV-PLSR scheme can be updated iteratively to include additional biologically relevant analytes, changes in pH, and interferents. Using Bayesian optimization, we introduce a pipeline for efficient optimization of multi-analyte, fit-for-purpose waveforms and machine learning approaches to voltammetric data analysis.

OPTIMISING FAST SCAN CYCLIC VOLTAMMETRY ANALYSIS FOR THE DETECTION OF DOPAMINE IN THE ZEBRAFISH BRAIN

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Keywords : Voltammetry, Dopamine, Zebrafish, MATLAB, Neurotransmitters

Detecting the release and reuptake of dopamine in the adult zebrafish (Danio rerio) brain presents unique challenges. The cyclic voltammogram (CV) is a visual representation of the oxidative and reductive profile of the electroactive substance released due to stimulation. Neurotransmitters such as dopamine have unique and recognisable CVs allowing them to be identified. Following electrical stimulation, optimised for the detection of dopamine, the adult zebrafish brain produces a CV that is dopamine like but appears to have other contributors to the profile. This work evaluated the ability of a calibration set of cyclic voltammograms at a variety of concentrations to resolve the neurochemical composition of solutions via principal component regression analysis. The constructed training set was evaluated for robustness and meta-analysis using rodent data was also conducted as a proof of principle step. The principal component regression resolved the changes in dopamine, pH, 5-HT, and histamine release evoked following electrical stimulation. The covarying nature of the code outputted concentrations relates to the electrically evoked nature of the neurotransmitter release, in essence a simulated environment. Further teasing out of the results in detail, particularly the response of dopamine compared to 5-HT in response to dopamine reuptake inhibitors allows further exploration of the robustness of this analysis method. The results of the analysis of histamine raises further questions about expanding and tailoring the calibration set depending on the biological model in question, however the results display that the resolution of overlapping cyclic voltammograms is possible with an appropriate calibration (training) set.

IN VIVO CHARACTERIZATION OF DOPAMINE SIGNALS BETWEEN STRIATAL SUBREGIONS IN FOUR DIFFERENT BEHAVIORAL PARADIGMS

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Keywords : dopamine, striatum, behavior, basal ganglia, functional topography

Dopamine signaling within the striatum has been critically implicated in processes such as reward, motivation, movement, and learning. A prevailing theory suggests that reward-related dopamine signals are uniformly broadcasted across the striatum, however, there is increasing evidence showing that dopamine signals are regionally heterogenous. In the present study, we assessed the regional coordination of striatal dopamine-release dynamics using chronically implanted microelectrodes for fast-scan cyclic voltammetry in the ventromedial (limbic) striatum (VMS), dorsomedial (associative) striatum (DMS), and dorsolateral (sensorimotor) striatum (DLS). We compared changes in extracellular dopamine concentration between striatal subregions on a trial-by-trial basis, and across four different behavioral paradigms that tested habitual behavior, time-tracking, appetitive and aversive Pavlovian conditioning, and cocaine self-administration. Our results show that dopamine release was consistently largest in VMS, followed by DMS and DLS. Event-related phasic dopamine signals in each trial of the behavioral tasks were classified as increase (+), decrease (-), or no change (0) compared to pre-event baseline concentration. Unexpectedly, instead of a highly reliable signal class across similar trials, we observed a mix of all signal classes. However, the average proportion of each classified signal class was stable across trials. Secondly, we observed an above-chance level occurrence of +/+ and -/- dopamine signals between two simultaneously recorded regions (i.e., region 1/region 2), suggesting that these regions had coordinated dopamine signals. Despite this coordination, the frequency of coordinated signals was below what would be expected of a uniform dopamine signal (i.e., 100% versus the observed 5-75% coordination). Lastly, to probe the behavioral function of signal coordination, we assessed whether coordinated signals were related to task performance. Overall, our analyses reveal that dopamine signals vary on a trial-by-trial basis, and are not uniform throughout the striatum but not completely uncoordinated.

ELECTROCHEMICAL DETECTION OF BEHAVIORALLY-EVOKED DOPAMINE RELEASE BY SUGAR FEEDING IN ADULT DROSOPHILA MUSHROOM BODY

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Keywords: *Drosophila melanogaster*, *In Vivo* Fast-Scan Cyclic Voltammetry, Behaviorally-evoked dopamine release, Sugar feeding, Mushroom Body

Drosophila melanogaster, the fruit fly, is a versatile model organism for studying the effects of genetic mutations on neurological processes. The dopaminergic system in *Drosophila* mushroom body (MB) has been extensively studied as an associative center for controlling olfactory learning and memory. However, there have not been methods to track dopamine concentration in real time during behavior. Our lab developed dopamine measurements using fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes in dissected out *Drosophila* brains. Here, we developed *in vivo* method based on FSCV to measure dopamine release in the MB during behavior for the first time. A customized chamber was employed to facilitate FSCV measurement in a fly that was immobilized but could freely move to perform sugar feeding. First, we characterized dopamine release *in vivo* for the first -time using acetylcholine stimulation. Application of 0.2 pmol acetylcholine stimulated 0.29 ± 0.06 μM dopamine in the MB medial tip. Nisoxetine, a dopamine transporter (DAT) inhibitor, and flupentixol, a D2 antagonist, increased stimulated release. Then, we monitored changes in behaviorally evoked dopamine release during sugar feeding. Sugar feeding evoked 0.31 ± 0.09 μM dopamine in the medial tip of MB. Flupentixol and nisoxetine significantly increased sugar-evoked release, implying that D2 receptors and DAT regulate dopamine signaling during sugar feeding. Therefore, these results show that sugar feeding causes a robust release of dopamine in the MB medial tip, similar to that of exogenous acetylcholine stimulations. FSCV is useful for measuring real-time changes in behaviorally-evoked neurotransmitters in the fly. This study provides a great addition to existing tool for quantifying *in vivo* dopamine and could be used to investigate variations in behaviorally evoked release in genetic mutant fly.

CHANGES IN DOPAMINE DYNAMICS AFTER ABSTINENCE FROM CHRONIC ETHANOL EXPOSURE IN MICE DORSAL AND VENTRAL STRIATUM

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Keywords: dopamine, striatum, ethanol exposure

Dopamine inputs to the dorsal and ventral striatum are critical for learning and alcohol motivated behaviors, respectively. Acute ethanol exposure activates mesolimbic dopamine (DA) neurotransmission, whereas withdrawal from chronic ethanol promotes decreases in ventral tegmental area DA neuron activity and extracellular DA levels in the nucleus accumbens (NAc). These findings suggest that chronic ethanol exposure may cause mesolimbic DA hypofunction, a condition significant for maintenance of alcohol use disorder by promoting ethanol consumption to compensate for its decreased DA release. Adult male and female mice were chronically exposed to ethanol using a voluntary consumption model (drinking in the dark) or a passive consumption model (ethanol vapor chambers). After 7 days of abstinence, ex vivo fast scan cyclic voltammetry in the dorsomedial striatum and NAc was performed to examine changes in DA terminal neurotransmission. Chronic ethanol exposure (CEE) followed by abstinence significantly increased evoked DA release and DA uptake rate in the dorsomedial striatum compared to control mice. In addition, mice exposed to CEE are more sensitive to the effects of ethanol 40 mM in the DMS than control mice. These data suggest that the persistence of DA uptake changes may have implications for vulnerability to relapse and other symptoms associated with prolonged ethanol abstinence such as alcohol-related sleep disruptions.

IN VITRO CHARACTERIZATION OF AN ELECTROCHEMICAL TECHNIQUE TO DETECT AMBIENT BRAIN HISTAMINE

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Keywords: Histamine, Serotonin, Fast scan cyclic voltammetry, Fast scan controlled adsorption voltammetry, Neuroinflammation

The brain is a dynamic mosaic of circuits that communicate via neurotransmission. Serotonin is a monoamine, believed to be implicated in depression. To study real-time neurotransmission in the brain, fast-scan cyclic voltammetry (FSCV) is employed to study the stimulated release of neurotransmitters, and fast-scan controlled adsorption voltammetry (FSCAV) to look at the ambient levels of neurotransmitters. These techniques utilize carbon-fiber microelectrodes (CFMs), which are biocompatible and cause minimal tissue damage to the surrounding area. Histamine is an important neuromodulator that has been shown to regulate the levels of serotonin in the brain. Recently, the Hashemi lab discovered that increased inflammation causes serotonin levels to drop significantly in the mouse hippocampus. While serotonin FSCAV has been established, histamine FSCAV has proven to be more difficult to generate. This is likely due to the kinetics of histamine oxidation. In addition, the complex brain matrix can convolute the signal. In this work we establish histamine FSCAV via waveform and electrode surface modifications. We discuss how a combination of changing waveform limits and scan rates, as well as novel polymers on electrodes, enables the first FSCAV measurements of ambient histamine.

AN ELECTRICAL APPROACH FOR SELECTIVE AND SENSITIVE DETECTION OF OPIOID PEPTIDES

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Keywords : electrochemistry, voltammetry, opioids, dynorphin

The endogenous opioid peptide systems are critical for analgesia, reward processing, and negative affect, but research on their *in vivo* function has been challenging due to an inability to detect changes reliably and consistently in opioid peptides. Thus, our work aims to develop innovative approaches for rapid and sensitive detection of opioid peptide release *in vivo*. Here, we have developed microimmunoelectrodes (MIEs) for electrochemical detection of opioid peptides using square-wave voltammetry. Briefly, a voltage is applied to the electrode to cause oxidation of the tyrosine residue on the opioid peptide of interest, which is detected as current. To provide specificity to these voltammetric measurements, the carbon fiber surface of the MIE is coated with antibody selective to the opioid peptide of interest. To test the sensitivity of the MIEs, electrodes are immersed in solutions containing different concentrations of opioid peptides and oxidative current is measured. Here, we show that dynorphin antibody-coated electrodes are sensitive to increasing concentrations of dynorphin in the attomolar range. To confirm specificity, oxidative current is also measured from exposure to tyrosine and other opioid peptides in solution. Our data show that dynorphin antibody-coated MIEs are sensitive and selective for dynorphin with little to no oxidative current observed in metenkephalin and tyrosine solutions. Future work aims to demonstrate the utility of these MIEs both *in vitro* via brain slice preparation and *in vivo* for real-time, rapid detection of endogenous opioid peptide release in awake and behaving animals.

METHAMPHETAMINE AND FENTANYL CO- SELF-ADMINISTRATION MODIFIES FENTANYL TAKING AND EXACERBATES MESOLIMBIC DOPAMINE DEFICITS

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Keywords : Polysubstance use, dopamine, fast scan cyclic voltammetry, kappa opioid receptors

Due to the recent increases in concurrent methamphetamine and fentanyl use and fentanyl-associated overdose deaths, it is vital to examine the interactions between these two substances. Male and female Long Evans rats were trained to self-administer 2.5 µg/kg/inf fentanyl. Following acquisition (2 consecutive days of 20 infusions), rats were randomly assigned to either fentanyl alone or fentanyl + methamphetamine and were tested on a short access, fixed ratio 1 schedule of reinforcement (3 hr sessions, max. 20 infusions) for ascending doses of fentanyl or combined fentanyl + methamphetamine (1.25, 2.5, 5.0 µg/kg/inf fentanyl ± 0.1 mg/kg/inf methamphetamine, 5 days per dose). Following self-administration, coronal brain slices containing the nucleus accumbens were prepared for ex vivo fast scan cyclic voltammetry. Rats self-administering the combination of fentanyl and methamphetamine completed their sessions more quickly, and had shorter latency to initiate responding than animals administering fentanyl alone at moderate and high doses of fentanyl. Combined fentanyl and methamphetamine rats had decreased evoked dopamine release and uptake rate (Vmax) compared to saline and fentanyl alone animals. Further, evoked dopamine release was decreased across stimulation amplitudes (1.4-10V) and in response to stimulation trains (5 pulses; 5, 10, 20, and 100Hz) in combined fentanyl and methamphetamine animals, compared to fentanyl alone animals. Together, these results highlight the complexities of combined opioid and stimulant use, and suggest that there may be unique neuroadaptive processes specific to combined fentanyl and methamphetamine which are not sufficiently explained by the individual changes observed following use of fentanyl or methamphetamine alone.

EXPLORING HOW PRESYNAPTIC CHOLINERGIC INPUT MODULATES DOPAMINE RELEASE IN MICE AND DROSOPHILA MELANOGASTER

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Keywords : dopamine, acetylcholine, GRAB sensors, FCV

In the striatum, Dopamine (DA) plays key roles in reward prediction signalling and goal-directed behaviour. In vivo in mammals, when DA neurons (DANs) increase activity in response to unexpected rewards or cues predictive of reward, there are synchronised pauses in activity of striatal cholinergic interneurons (ChIs). Acetylcholine (ACh) released from ChIs modulates DA release through nicotinic acetylcholine receptors (nAChR) located on striatal DA axons. Ex vivo studies using fast-scan cyclic voltammetry (FCV) have shown that nAChRs enhance DA release during low frequency stimulation but decrease it during high frequency stimulation, suggesting that ChI pauses might promote reward-related signalling. However, the downstream mechanisms by which nAChRs gate these DA release dynamics, and what this signal codes for, are not well understood. The Drosophila mushroom body (MB) is a centre for olfactory learning that also has pertinent ACh-DA interactions. Reciprocal synapses between cholinergic Kenyon Cells (KCs) that provide odour identity, and DANs that provide valence to an odour during learning, have been shown to be involved in aversive learning through cholinergic modulation of DA release through nAChRs. The precise dynamics between the two neurotransmitters remain to be established as neither firing nor release patterns from KCs are well understood, and DA release has never been studied directly. We are exploring the potentially conserved parallels and mechanisms underlying the ACh regulation of DA release in flies and mouse, as well as DA and ACh dynamics in flies during behaviour. To understand DA and ACh dynamic in behaving flies, we imaged MB neurones expressing GRAB sensors in tethered Drosophila. In parallel, we used FCV and GRAB sensors in mouse brain slices to monitor DA and ACh respectively to investigate molecular mechanisms downstream of nAChR activation, including voltage-gated ion channels. We will report on our findings to date. Our inter-phylum approach could uncover conserved mechanisms and common function of cholinergic modulation of DA release by linking molecular mechanisms to the behaviourally relevant signals.

LONG-LASTING ALTERATIONS IN AXONAL DOPAMINE RELEASE REGULATION & UPSTREAM TRANSCRIPTION INDUCED BY ETHANOL DRINKING IN MACAQUES

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Keywords: Kappa opioid receptor, mesolimbic dopamine, fast scan cyclic voltammetry, ethanol, rhesus macaque

Though alcohol use disorder is prevalent across the globe, pharmacological treatments still have limited efficacy and relapse rates remain high. A critical component in the development of substance use disorders is experience-dependent plasticity in the mesolimbic dopamine system, which is composed of dopamine projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). The kappa opioid receptor (KOR) system is a dynamic regulator of dopamine release in the NAc, and KOR antagonism reliably shows therapeutic promise for alcohol use disorders. The prevailing theory of KOR involvement in substance use posits that experience-dependent upregulation of the KOR system decreases dopamine release, thus driving relapse after periods of abstinence. However, minimal work has been conducted in non-human primates – a critical next step for advancing therapeutic endpoints and furthering our understanding of primate neurophysiology. Here, we investigate the interplay of KOR activity and dopaminergic function in rhesus macaques following 18 months of ethanol self-administration and three 1-month forced abstinence periods. Following the final 1-month abstinence period, subjects were sacrificed for *ex vivo* fast-scan cyclic voltammetry recordings in NAc and RNA sequencing of VTA. In the NAc core, elicited dopamine release was increased by KOR antagonism to a greater extent in drinkers compared to controls, indicating augmented inhibitory feedback via release of endogenous dynorphins. Application of a KOR agonist revealed marked upregulation of KOR inhibition of dopamine release in drinkers, demonstrating that augmented KOR sensitivity persists well into abstinence. Paired with RNA-seq data from the VTA of the same animals, we define the upstream transcriptional signatures that may mediate the long-lasting alterations in KOR function. Together, this data demonstrates prolonged upregulation of KOR-mediated inhibition of dopamine release both through increased endogenous activity and response to exogenous ligand. This work brings a new understanding of circuit regulation and gene expression associated with voluntary alcohol use in a non-human primate model.

REDUCED DOPAMINE TERMINAL FUNCTION IN THE NUCLEUS ACCUMBENS FOLLOWING HEROIN SELF-ADMINISTRATION

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Keywords : Dopamine, Heroin, Fast-scan Cyclic Voltammetry, Microdialysis, Nucleus Accumbens

Recent evidence has illustrated that heroin produces its rewarding and addictive effects, at least partially, through activation of the mesolimbic dopamine system. Notably, it has been shown that heroin seeking can be attenuated by systemic administration of antagonists of negative regulators of the dopamine system, such as kappa opioid receptors (KOR) or presynaptic D3 receptors (D3R), thus increasing dopamine levels. Therefore, it is likely that withdrawal from chronic heroin exposure drives a state of hypodopaminergia in the nucleus accumbens (NAc), as previously observed following withdrawal from chronic stimulant and alcohol use. To this end, this study aimed to (1) investigate alterations in the dopamine terminal function following heroin self-administration in male and female rats and (2) identify a mechanism for hypodopaminergia following chronic heroin self-administration. Adult male and female Long Evans rats were trained to self-administer heroin (0.05 mg/kg/inf) and then placed on long access (LgA; FR1, 6-hr session, unlimited infusions, 0.05 mg/kg/inf) heroin self-administration paradigm to induce escalation of heroin intake. Following LgA, rats were utilized for neurochemical analyses – in vivo microdialysis and ex vivo fast-scan cyclic voltammetry (FSCV) in the NAc. The results support previous literature in that following LgA, male and female rats had decreased basal extracellular levels of dopamine as well as a reduced dopaminergic response to a heroin challenge (0.1 mg/kg/inf, IV) in the NAc during withdrawal from heroin self-administration. FSCV results revealed that heroin exposed rats have reduced dopamine release during single-pulse stimulations but increased phasic-like dopamine release during stimulation trains (5 pulses, 5-100Hz) when compared to their heroin naïve counterparts. In addition, we found that presynaptic D3R and KOR activity in the NAc was increased following LgA in male and female rats. These results reveal a marked reduction in dopamine system function following heroin exposure and identify a potential mechanism in the NAc that may drive the hypodopaminergic state observed during heroin withdrawal.

REAL TIME MEASUREMENTS OF DOPAMINE AND GLUTAMATE IN RAT STRIATUM USING FAST-SCAN CYCLIC VOLTAMMETRY

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Keywords: Glutamate, dopamine, FSCV, biosensors, striatum

Glutamatergic and dopaminergic transmission within the striatum is interrelated and linked to several psychiatric, neurodevelopmental, and neurodegenerative pathologies. Investigation of the modulatory action of dopaminergic neurons in the mesolimbic and nigrostriatal pathways on striatal glutamate, and the reciprocal modulation of striatal dopamine (DA) through glutamate receptors on DA terminals has been limited due to experimental techniques lacking temporal resolution. The release and removal of neurotransmitters from the synapse occurs within milliseconds; thus, analytical techniques capable of monitoring rapid neurochemical fluctuations on this timescale are required in order to directly quantify these processes. Fast-scan cyclic voltammetry (FSCV) is an electroanalytical technique with superb temporal resolution that is commonly used to monitor rapid DA fluctuations, even in the presence of interferents. FSCV is typically coupled with carbon-fiber microelectrodes. These can be modified with a chitosan matrix containing an oxidase enzyme to allow for the simultaneous detection of non-electroactive analytes. In this work we have modified the carbon-fiber microelectrodes with glutamate oxidase (GlutOx) to allow for the detection of glutamate in live rat brain tissue. The GlutOx-modified electrodes have been characterized for their stability, selectivity, oxygen dependency, and sensitivity to glutamate and DA. Additionally, glutamate has been detected in rat striatal tissue upon electrical stimulation using an *ex-vivo* brain slice preparation. The signal was verified using the pharmacological agent, DL-TBOA, a potent and selective inhibitor of the excitatory amino acid transporter that is responsible for glutamate reuptake. Future work will entail characterization of the DA and glutamate kinetics in intact, anesthetized animals via stereotaxic surgery. The ability to simultaneously record rapid fluctuations of glutamate and DA at one electrode promises to inform improved therapeutic strategies for a wide range of disorders in which both dopaminergic and glutamatergic signalling are altered, including Parkinson's Disease, addiction, and Huntington's Disease.

LIGHT STIMULATION REDUCTION IN NEURONAL ACTIVITY IS DEPENDENT ON THE TEMPORAL PATTERN AND LIGHT POWER USED

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Keywords: Electrophysiology, optogenetics, neuronal activity

Neuronal sensitivity to light stimulation can represent a serious confounding factor for those assays using light to investigate neuronal processes such as optogenetics and optical imaging. Here we studied the effects produced by ten different patterns of light stimulation delivered to different populations of brain neurons. We showed that light in the visible spectrum produces an outward hyperpolarizing current and an inhibition of neuronal activity, which increases with the light duty cycle, pulse duration and power used. Importantly, these effects are correlated with the light-induced increase in tissue temperature. Overall our results provide a guideline to avoid artefactual effects when applying experimental protocols based on brain light stimulation.

INVESTIGATING THE REGULATION OF STRIATAL DOPAMINE RELEASE BY STRIATAL NOREPINEPHRINE AT ADRENERGIC RECEPTORS

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Keywords: Dopamine, Noradrenaline, Striatum, GRAB sensors

Release of dopamine (DA) in the striatum is critical to the regulation of action selection and motivation. DA neurons have extensively branched axons, which form a major site for the regulation of DA release by local axonal mechanisms and striatal neuromodulators. The striatum is sparsely innervated by norepinephrinergic neurons originating in the locus coeruleus and ventral norepinephrinergic bundle and evidence suggests norepinephrine (NE) might regulate DA release in the striatum via adrenoreceptors. However, it is unresolved whether the effects of NE on striatal DA release are mediated by direct actions on DA axons or indirectly via other cells, such as cholinergic interneurons or astrocytes. Here, we investigated the regulation of DA transmission in the striatum by NE receptors and their underlying circuits.

We detected electrically evoked DA release at carbon-fibre microelectrodes using fast-scan cyclic voltammetry in mouse striatal slices following the pharmacological manipulation of NE receptors. The a1 agonist phenylephrine decreased DA release evoked by single electrical pulses by ~30-40% in the caudate putamen (CPu) and nucleus accumbens core (NAc), and modulated the activity-dependence of DA release in NAc but not CPu. We are currently pursuing the mechanisms and circuits responsible, as well as the impact of b adrenoreceptors. Additionally, we are exploring striatal NE dynamics by imaging the genetically-encoded GPCR activation-based NE sensor, $GRAB_{NE2h}$, in striatal slices. $GRAB_{NE2h}$ fluorescence was responsive to exogenous NE but not DA in a concentration-dependent manner, and reported rapid electrically evoked transients. We are characterising the contribution to these signals of endogenous NE versus DA, with a view to further exploring striatal NE signalling and its regulation. Overall, we aim to increase understanding of the role of striatal NE in the regulation of DA transmission using novel tools to provide high temporal and spatial resolution of neurotransmitter release.

GESTATIONAL ETHANOL EXPOSURE INDUCES SEX-SPECIFIC IMBALANCES IN STRIATAL ACETYLCHOLINE AND DOPAMINE DYNAMICS

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Keywords: ethanol, dopamine, acetylcholine, photometry, voltammetry

Fetal Alcohol Spectrum Disorder (FASD) is a condition that occurs in a child exposed to alcohol before birth, leading to harmful consequences on cognition and motor skills and these sequelae are recapitulated in animal models of gestational ethanol exposure (GEE). Deficits in striatal cholinergic and dopamine (DA) function impair action learning and execution; however, the effects of GEE on striatal acetylcholine (ACh) and DA remain unexplored. Here, we report that ethanol exposure during the first ten postnatal days ($GEE^{P0\text{-}P10}$) induces sex-specific anatomical and motor learning deficits in female mice. Voltammetry experiments show that these behavioral deficits are associated with increased DA levels in the dorsolateral striatum (DLS) of $GEE^{P0\text{-}P10}$ female, but not male mice. In addition, female mice display impaired striatal β2 nicotinic acetylcholine receptor (nAChRs)-modulation of electrically evoked DA release as the selective β2-subunit nAChR antagonist DhβE induces a larger decrease of DA transients evoked by single-pulse electrical stimulation in female $GEE^{P0\text{-}P10}$ compared to female control mice, pointing to increased nAChR function to drive DA release. Conversely, DhβE fails to increase DA during bursts of electrical stimulation (100 Hz) in $GEE^{P0\text{-}P10}$ female compared to control animas, indicative of nAChR desensitization. To investigate whether striatal DA deficits were due to impaired ACh release we used the fluorescent sensor $GACh_{3.0}$ expressed in the DLS of $GEE^{P0\text{-}P10}$ and control female offspring. Using *in vitro* photometry, we monitored changes in dF/F amplitude induced by single-pulse and 6-pulse 100 Hz stimulation, before and after bath application of the acetylcholinesterase inhibitor, galantamine. While galantamine did not affect the amplitude of dF/F transients induced by 1 pulse stimulation, it increased the amplitude of dF/F transients evoked by 100 Hz stimulation in control and $GEE^{P0\text{-}P10}$ females and revealed a faster ACh transient decay in $GEE^{P0\text{-}P10}$ compared to control females. Altogether these experiments highlight novel mechanisms of striatal dysfunction involving abnormalities in balance between ACh and DA dynamics in a mouse model of FASD.

CONVERGENT REGULATION OF PRESYNAPTIC SHORT-TERM PLASTICITY IN STRIATAL DOPAMINE RELEASE BY DOPAMINE TRANSPORTERS AND GABA RECEPTORS

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Keywords: Dopamine, dopamine transporter, GABA receptor, short-term plasticity, striatum

Axonal dopamine (DA) release within the striatum is not an accurate reflection of action potential activity – rather, it exhibits short-term plasticity, ranging from short-term facilitation at short inter-pulse intervals, to short-term depression at longer intervals. Both the DA transporter (DAT) and γ-aminobutyric acid receptors (GABA-Rs) have been shown to modulate this plasticity. The DAT in particular, alongside its roles in mediating DA uptake and apparently gating mobilisation of vesicle pools, has emerged as a strong regulator of this short-term plasticity. The DAT is electrogenic, with transport of DA associated with membrane depolarisation, which limits axonal re-activation and drives release-independent depression, and also strongly supports the frequency dependence of DA release. GABA provides an endogenous GABA tone at GABA-Rs that limits DA release, with $GABA_A$-Rs known to limit action potential propagation and spike amplitude via a paradoxical depolarisation inactivation. Despite limiting DA signal amplitude, GABA-Rs also slightly supports the frequency dependence of DA release.

We assessed whether there is a co-operation or interdependence between these mechanisms in supporting the dynamics of short-term plasticity in DA release, by using fast-scan cyclic voltammetry to detect DA evoked by electrical stimulation in mouse striatal slices. We found that GABA antagonists attenuated the effect of DAT inhibition by cocaine on DA release evoked by single pulse stimulation. Further, using paired-pulse stimulation paradigms, with inter-pulse intervals equivalent to 5-100Hz firing frequency, GABA-R agonists limit the effects of cocaine on short-term facilitation. These results suggest a convergence or co-operation between DAT function and GABA tone acting at GABA-Rs in determining the relationship between striatal dopamine axon activity and exocytosis, that together support the frequency dependence over absolute amplitude of DA signalling.

CHARACTERIZING SEROTONIN SIGNALING IN HUMAN EPIDERMAL CELLS WITH FAST SCAN CYCLIC VOLTAMMETRY

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Keywords : Executive functions, Polyunsaturated Fatty Acids, Dopamine, D-light,

Depression is a neuropsychiatric disease that affects over 250 million people worldwide. Currently there are no chemical diagnostic tools available to aid physicians in choosing a personalized course of treatment. Low levels of brain serotonin are implicated in the pathophysiology of depression, but a peripheral biomarker has not yet been identified as serotonin levels in the brain are not reflected in the rest of the body. We have found ample evidence in the literature showing that serotonin, along with its cellular machinery are present in skin and hair cells. As such, we hypothesize that probing these skin cells could be an easily accessible, non-invasive strategy for defining serotonin chemistry in the body, as a potential translational tool. Here, we utilized fast scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes to measure real-time serotonin signaling in epidermal cell types: bulge cells, keratinocytes and fibroblasts for the first time. As these cell types have not been previously probed with FSCV, we first established and optimized experimental protocols to facilitate sensitive serotonin measurements. Then we applied a mechanical stimulation paradigm with varying intensities to each cell type to induce serotonin release. We found all cell types to consistently release serotonin both spontaneously and in response to stimulation. This novel finding presents skin cells as a viable chemical system for probing serotonin. With this exciting work we will continue to investigate the potential of this easily accessible model system as a potential translational tool in humans and explore the connection between skin signaling and mood disorders.

SYNAPTOGYRIN-3 PREVENTS COCAINE-INDUCED BEHAVIORAL AND DOPAMINERGIC ADAPTATIONS

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Keywords : Fast scan cyclic voltammetry, Dopamine Transporter, Synaptogyrin-3, Dopamine, Cocaine

Synaptogyrin-3 (SYG3) is a synaptic vesicle protein highly expressed in dopamine-containing neurons that directly interacts with the dopamine transporter (DAT), suggesting a role in synaptic dopamine dynamics. The DAT is the primary reinforcing site of cocaine, and chronic cocaine exposure alters DAT function, expression, and dopamine release dynamics. We tested the hypothesis that chronic cocaine exposure disrupts SYG3 function, resulting in DAT alterations that drive excessive cocaine taking. Rats were trained to self-administer cocaine and tested on a progressive ratio (PR) schedule of reinforcement. Western blots showed a significant positive correlation between SYG3 and DAT protein levels and a significant negative correlation between SYG3 and PR breakpoint in the ventral tegmental area and nucleus accumbens. Thus, we virally-overexpressed SYG3 in VTA dopamine neurons of cocaine-naive rats to assess the effects on cocaine and sucrose self-administration, anxiety-like behavior, and cognition tests. Additionally, nucleus accumbens dopamine terminal function was measured using ex vivo fast-scan cyclic voltammetry. SYG3 overexpression resulted in reduced cocaine responding on an extended access schedule and decreased PR breakpoint. SYG3 overexpression also reduced anxiety-like without altering sucrose self-administration, sucrose preference, or pairwise discrimination. While SYG3 overexpression did not affect dopamine uptake rate in cocaine-naive rats, it bidirectionally altered dopamine release—blunting release in response to high stimulation intensities but increasing release at low stimulation intensities. Together, these data provide evidence for SYG3's role as a regulator of dopamine kinetics and a potential target for pharmacotherapeutics to treat cocaine use disorder.

ATTENUATION OF STIMULATED ACCUMBAL DOPAMINE RELELASE BY NMDA IS MEDIATED THROUGH METABOTROPIC GLUTAMATE RECEPTORS.

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Dopamine, Nucleus accumbens, NMDA, mGluR, Voltammetry

Changes in glutamate regulation of dopamine release in the mesolimbic pathway, projecting from the ventral tegmental area to the nucleus accumbens (NAc), are implicated in schizophrenia. In particular NMDA-mediated mechanisms are involved: NMDA antagonists exacerbate symptoms in patients, and provide a robust model of schizophrenia in rodents. In this context, the recent observation that NMDA caused an attenuation of electrically-stimulated dopamine release in NAc may be relevant to understanding the mechanism of this regulation. However, this action of NMDA on dopamine release is unlikely to be direct, since (1) NMDA receptors are excitatory and (2) evidence suggests that there are no NMDA receptors on dopamine terminals in this region. Therefore, the action of NMDA is likely to be through an intermediary mechanism, of which the most likely candidates are acetylcholine, GABA, or metabotropic glutamate receptors (mGluR).

To identify the intermediate mechanisms, fast-scan cyclic voltammetry was used to measure the effects of cholinergic, GABA-ergic and mGluR antagonists on the attenuation of electrically-stimulated (10 x 1ms pulses; 60Hz; 800µA) dopamine release caused by NMDA in NAc of rat brain slices *in vitro*. Recordings were taken every three minutes during baseline (4 stimulations), drug (4 stimulations), and washout (6 stimulations).

NMDA (30µM) attenuated stimulated dopamine release in the NAc, replicating previous findings. Neither nicotinic (DhβE; 10µM) or muscarinic (scopolamine; 1µM) cholinergic antagonists nor GABA-A (picrotoxin; 100µM) or GABA-B (CGP54626; 1µM) antagonists blocked the NMDA attenuation of dopamine release. However, the group II mGluR antagonist (MCPG: 100µM) did reverse the attenuation of stimulated release caused by NMDA. This demonstrates that the modulatory action of NMDA on dopamine release in the NAc is mediated through group II mGluR, but not through cholinergic or GABA-ergic mechanisms. This has implications for understanding glutamate/dopamine dysregulation in schizophrenia

STRIATAL ACETYLCHOLINE REPORTS DISTINCT UPDATE SIGNALS DURING FLEXIBLE MULTI-STEP DECISION MAKING

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Keywords: Acetylcholine, Striatum, Decision Making, Behavior, Reward

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

LOCAL METABOLIC REGULATION SUSTAINS COLLATERAL AXON BRANCHING OF CORTICAL NEURONS

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Keywords: Biosensors, Fluorescence based-imaging

Aim:
The brain is one of the most energetically demanding organs in the body. Yet the molecular mechanisms adapting energy production to local metabolic activity still remain only partially understood. Previous data from our lab demonstrated that mitochondria are distributed along axons and captured at immature presynaptic sites under the control of the AMPK-related kinase NUAK1. This phenomenon is necessary for axonal branching in developing cortical neurons. However, the precise mechanism explaining how mitochondria support axon branch formation is unknown.
Method:
To better characterise local metabolite production and consumption occurring at different levels of the axon, we used genetically-encoded fluorescent tools with high spatial and temporal resolutions to assess metabolites used at a subcellular level and in real time.
Results:
We demonstrated that the lack of NUAK1 leads to metabolic alterations specifically at the mitochondrial level. We identified that NUAK1 acts through the regulation of the expression of genes linked to mitochondrial function. Restoration of mitochondrial function in NUAK1 null neurons rescues collateral formation.
Conclusion:
Altogether, our findings contribute to the growing evidence that mitochondrial-depend remodelling of local metabolic state is critical for axonal morphogenesis and normal brain development.

ALTERATIONS IN STRIATAL CHOLINERGIC INTERNEURONS AND ACETYLCHOLINE RELEASE IN A PARKINSON'S DISEASE MOUSE MODEL

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Keywords : Striatum, Parkinson's disease, Cholinergic interneurons, Acetylcholine, Fluorescent sensors

The complex and reciprocal interactions between striatal dopamine (DA) and acetylcholine (ACh) neurotransmitter systems have been heavily implicated in Parkinson's disease (PD). Adaptations in cholinergic systems in PD have been suggested to occur and to result in striatum in a hypercholinergic state. However, most prior work has relied on acute toxin models, which is not representative of the slower, progressive, system-wide phenotype seen in human patients. We assessed whether there are alterations to striatal ACh signalling in a slowly progressive transgenic mouse model of early parkinsonism, the human wild-type alpha-synuclein overexpressing (*SNCA*-OVX) model. This model shows mild deficits in DA release in dorsal but not ventral striatum from a young age and develops mild DA neuron loss and motor deficits in old age.

Using immunohistochemical approaches, we assessed numbers of cholinergic interneurons (ChIs) and their basal activity by labelling for respectively choline acetyltransferase and phosphorylated ribosomal protein S6 (a marker for basal neuronal activity in ChIs). We found that *SNCA*-OVX animals at 3-4 months have greater ChI density but lower basal activity in comparison to background control animals (alpha-synuclein-null mice). We then assessed basal and evoked levels of ACh release in the dorsal striatum of *SNCA*-OVX mice using the genetically encoded fluorescent ACh sensor $GRAB_{ACh3.0}$. We found a higher $GRAB_{ACh}$ basal fluorescence in *SNCA*-OVX compared to control mice, indicative of a higher tonic level of striatal ACh. Local electrical stimulus trains (1-5 pulses, 2, 5, 10Hz) evoked rapid ACh transients that summated to higher levels in *SNCA*-OVX than control mice. Furthermore, we found that ACh release in SNCA-OVX mice was more strongly modulated by D2-DA receptors, while GABAergic tonic inhibition of ACh release seemed unaltered. These findings reveal multiple alterations to the striatal cholinergic system in a physiological mouse model of early PD, that indicate a lower activity per ChI, but overall, a net elevation in levels of ACh release that support the hypothesis that in PD, the striatum is in hypercholinergic state.

DISTINCT COMPARTMENT-SPECIFIC PLASTICITY SIGNATURES IN MESOLIMBIC DOPAMINE ACROSS CONTINGENCY LEARNING

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Keywords: dopamine, somatodendritic release, optical imaging, fiber photometry, plasticity

Deficits in reward learning and motivation in diseases such as anxiety, depression, and substance use disorder have been directly linked to experience-induced alterations in dopamine transmission in the ventral tegmental area (VTA) to nucleus accumbens (NAc) pathway. A majority of the work outlining the underlying mechanisms of dopaminergic plasticity has centered on electrophysiology at cell bodies or release mechanisms at terminals, despite the fact that dopamine is released from multiple cellular compartments – including somatodendritic (sDA) release in the VTA. Moreover, it has been theorized that sDA release functions simply as a feedback mechanism to downregulate terminal activity during periods of heightened activity within this circuit. Here, we first utilized fiber photometry to record *in vivo* dopamine kinetics using the optical sensor, dLight1.2, concurrently in both the terminal and somatodendritic compartments during a discriminative learning operant task. Our results revealed distinct activity-dependent signatures across contingency learning in the two compartments, challenging the hypothesis that somatodendritic release merely tracks axonal release patterns. Next, we characterized several potential mechanisms for this compartment-specific plasticity using *ex vivo* fast-scan cyclic voltammetry (FSCV) and optical slice imaging methods paired with pharmacology at various points across the same learning task. Our results indicate enhanced high frequency evoked dopamine release in the NAc terminals in animals following contingency learning compared to naïve controls. In the VTA, the peak of evoked sDA release was unchanged in animals that underwent the task. However, our preliminary data indicate that unlike terminal release, sDA release is partially insensitive to VMAT-2 inhibition in naïve controls, and after learning release appears almost entirely independent of VMAT-2. Together, these data suggest multiple release mechanisms are involved in sDA release and may be differentially regulated by experience. Overall, the results of these experiments support temporally- and compartment-specific roles of dopaminergic plasticity in basal cognitive functions, which ultimately further extends our understanding of these processes in both health and disease.

EFFECTS OF DIETARY PROTEIN RESTRICTION ON NUCLEUS ACCUMBENS DOPAMINE MEASURED VIA FIBER PHOTOMETRY

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Keywords: protein restriction, fiber photometry, GRAB-DA, dopamine, nucleus accumbens

Neural activity in ventral tegmental area (VTA), measured via the calcium sensor GCAMP6s, is higher when protein-restricted rats drink a protein solution than when they drink a carbohydrate solution. The VTA is a major source of dopamine in the brain, and fast-scan cyclic voltammetry in brain slices shows greater stimulated dopamine release in nucleus accumbens (NAc) of protein-restricted rats than in non-restricted controls. Thus, the involvement of the mesolimbic dopamine system in protein restriction is clear. Nonetheless, in vivo measurement of dopamine itself – rather than dopamine neuron activity – in response to protein consumption has been missing. Here, we used fiber photometry to measure dopamine in NAc of protein-restricted (PR) and non-restricted (NR) mice drinking "Resource Complete", a high-protein (23%) mixed nutrient solution, and "Scandishake", a low-protein (4.9%) mixed nutrient solution. We found that PR mice drank significantly more Resource Complete than NR mice, but that intakes were equivalent when Scandishake was offered. Next, we stereotactically injected mice with the newly developed version of the fluorescent dopamine sensor $GRAB_{DA.2m}$ and implanted a 200 µM core fiber optic (length=5 mm) aimed at NAc lateral shell. We observed dopamine-driven changes in fluorescence in response to licking, however, neither the signal peak nor AUC differed between NR and PR mice drinking Resource Complete or Scandishake in different sessions. Ongoing experiments are investigating whether familiarization with each solution, i.e. allowing mice to associate the taste with its post-ingestive feedback, will alter dopamine release. Furthermore, we will test mice when given access to both solutions in the same session and have a choice of which solution to consume. Together, these experiments will provide information about how dopamine release in NAc is (or is not) affected by the state of protein restriction.

CONTRIBUTION OF DORSAL STRIATAL DIRECT AND INDIRECT PATHWAY PROJECTIONS TO GOAL-DIRECTED AND HABIT LEARNING

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Keywords: instrumental, calcium imaging, ensembles, miniature microscopy, striatum

Optimal behavior relies on a balance between two distinct strategies; one goal-directed in which the relationship between actions and their consequences is considered, and one habitual, which allows routine tasks to be conducted without forethought of their consequences. The balance between these systems allows adaptive and efficient behavior, but disruption of this balance can lead to symptoms characteristic of several psychiatric and neurological diseases. Goal-directed and habit learning are known to rely on the anatomically distinct dorsomedial (DMS) and dorsolateral (DLS) striatum, respectively. But very little is known about the subregion-specific contribution of the two major subtypes of striatal projection neurons (SPNs): the direct (dSPNs; characterized by D1 receptor expression) and indirect (iSPNs; characterized by A2A receptor expression) striatal projections neurons. Using chemogenetics and DRD1-cre and A2A-cre driver mice, we selectively inactivated dSPNs or iSPNs in either the DLS or DMS during instrumental lever press reward training. Following training, devaluation via sensory-specific satiety of the food outcome was used to probe goal-directed versus habitual control of instrumental behavior. The data show that in the pDMS both dSPNs and iSPNs mediate goal-directed learning. Chemogenetic inactivation of dSPNs or iSPNs during instrumental training produces insensitivity to devaluation following limited goal-directed training, while activation of either projection promotes goal-directed behavior even after extended habit training. Conversely, dSPNs, but not iSPNs, from the DLS are required to form behavioral habits. Ongoing experiments, using miniscopes to image calcium activity with cellular resolution in subregion- and cell-type-specific striatal projection pathways, are addressing how activity of each cell type reorganizes with the transition to habitual control.

NEAR-INFRARED FLUORESCENT NANOSENSORS FOR IMAGING OXYTOCIN RELEASE IN BRAINS

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Keywords : Oxytocin, Biosensor, Neuropeptide, Carbon Nanotube

Neuropeptide oxytocin plays essential roles in mammalian social and reproductive behavior. Released throughout the brain, oxytocin regulates complex emotions and social behaviors and has been implicated in the pathogenesis of various social impairment disorders such as autism spectrum disorder (ASD). While oxytocin has been studied extensively, we lack the tools to directly probe oxytocin signaling *in vivo* and fully elucidate its function. To address this need, we have developed and validated a nanosensor capable of imaging oxytocin release in the brain. Our nanosensor leverages the tissue-transparent near-infrared fluorescence of single-walled carbon nanotubes (SWCNT) and the sensing capacity of an oxytocin receptor peptide fragment (OXTp). We covalently conjugated OXTp to the SWCNT surface and adsorbed C_{12} DNA to this product to impart colloidal stability. The resulting conjugate forms our near-infrared oxytocin nanosensors (nIROx) that respond fluorescently to oxytocin with a $\Delta F/F_o$ >500% *in vitro.* Selectivity screening of nIROx revealed selectivity for oxytocin over its structural analogue, vasopressin, and various neurotransmitters and hormones, including thyrotropin-releasing hormone. We further demonstrated that nIROx respond reversibly to oxytocin and retain their sensitivity upon immobilization on a solid substrate. Following *in vitro* validation, we introduced nIROx into the hypothalamic paraventricular nucleus of acute mouse brain slices. nIROx respond immediately and reversibly to electrically stimulated oxytocin release with an integrated $\Delta F/F_o$ of up to 40%. In the presence of atosiban, a positive control shown to reduce nIROx response to oxytocin *in vitro*, nIROx response in brains decreases by up to 50%. Alternatively, nIROx retain their sensitivity in acute brain slices upon incubation with quinpirole, a dopamine receptor agonist. Taken together, these results suggest that nIROx response in brains is attributable to oxytocin release and insensitive to off-target pharmacological agents. The data summarized herein demonstrate that nIROx are selective and reversible nanosensors capable of imaging oxytocin release in brains.

A NEAR INFRARED FLUORESCENT SENSOR PAINT TO IMAGE DOPAMINE SIGNALING

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Keywords : Dopamine, fluorescence microscopy, sensors, exocytosis, signalling

Cells use molecules to exchange information. A very prominent example is neurotransmitter signaling between neuronal cells. The neurotransmitter dopamine is released from discrete axonal structures called varicosities. Its release is essential in behaviour and is critically implicated in prevalent neuropsychiatric diseases but existing dopamine detection methods are not able to detect and distinguish discrete dopamine release events from multiple varicosities. This prevents an understanding of how dopamine release is regulated across populations of discrete varicosities. Using a near infrared fluorescent (980 nm) dopamine nanosensor 'paint' (AndromeDA) based on single-walled carbon nanotubes (SWCNTs), we show that action potential-evoked dopamine release is highly heterogeneous across release sites and also requires molecular priming. Using AndromeDA, we visualize dopamine release at up to 100 dopaminergic varicosities simultaneously within a single imaging field with high temporal resolution (15 images/s). We find that 'hotspots' of dopamine release are highly heterogeneous and are detected at only ~17% of all varicosities. In neurons lacking Munc13 proteins, which prime synaptic vesicles, dopamine release is abolished during electrical stimulation, demonstrating that dopamine release requires vesicle priming. In summary, AndromeDA reveals the spatiotemporal organization of dopamine release.

DECIPHERING THE ROLE OF DOPAMINE IN THE MOUSE MEDIAL PREFRONTAL CORTEX

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Keywords: Mesocortical dopamine, prefrontal cortex, attention, fiber photometry

Midbrain dopaminergic (DA) projections to the medial prefrontal cortex (mPFC) exert a powerful neuromodulatory influence over the mPFC and evidence has linked dysregulation of this pathway to an array of neuropsychiatric disorders. In accordance, mPFC DA transmission has been implicated in a variety of behaviorally relevant processes including working memory, cognitive flexibility, and stress reactivity; however, relative to other brain regions whose activity is extensively modulated by midbrain DA systems, the role of mPFC DA transmission remains considerably less well understood. Until recently, investigations of mPFC DA have been prohibitively difficult and limited to either slow, direct measures of extracellular DA such as microdialysis or fast but non-selective measures such as fast-scan cyclic voltammetry. Here, we utilized the fluorescent DA sensor dLight, which has high specificity for DA and sub-second temporal resolution, in conjunction with fiber photometry to parse mPFC DA dynamics in freely behaving mice. Contrary to prior work suggesting that mPFC DA release is preferentially evoked by stressful events, we demonstrate robust DA signal to novel (auditory tone), appetitive (sucrose), and aversive (footshock) stimuli. DA signal to novel stimuli diminished across sessions while the magnitude of responses to sucrose and footshock were proportional to lick bout size and shock intensity, respectively. Interestingly, while we observed no cue-evoked DA signal during an operant task in which animals learned to initiate a correct response for sucrose during a prolonged (30sec) cue presentation period, in a similar task wherein the cue indicating the correct operant response was only presented briefly (1sec) following a variable delay, we observed a cue-evoked increase in DA signal that was preceded by a pronounced decrease in signal during the delay period and that this decrease was highly correlated with task performance. Together, these findings suggest that this dampening of mPFC DA activity serves to filter environmental stimuli to allow selective attention towards a specific expected stimulus, which triggers an mPFC DA transient when detected.

BETA-ARRESTIN INVOLVEMENT IN DIFFERENTIAL EFFECTS OF ACUTE STRESS ON ABETA LEVELS IN MALE AND FEMALE MICE

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Keywords: Alzheimer's disease, stress, sex-dependent, in vivo microdialysis

Almost 70% of people living with AD are female. Interestingly, stress-induced corticotrophin releasing factor receptors (CRF-Rs) signal differently in females and males. In females, CRF-Rs normally activate PKA/ERK. In males, CRF-Rs are withdrawn from the plasma membrane by beta-arrestin, resulting in significantly less CRF signaling. We hypothesize that the involvement of beta-arrestin in the stress signaling pathway in males underlies the differences in abeta levels in response to stress. We used in vivo microdialysis to measure brain ISF Abeta levels every hour for several hours before, during, and after acute restraint stress in living APP transgenic mice. To study the influence of beta-arrestin1 on stress-induced changes in Abeta, we measured the effects of acute stress on Aβ levels in male and female beta-arrestin1 knock-out mice. To elucidate the CRF-signaling pathways, we used CRF, PKA, and ERK inhibitors before acute stress exposure. In females, acute restraint stress causes a rapid increase in brain interstitial fluid (ISF) Aβ levels in the hippocampus, whereas Aβ in males does not change. The increase in females is blocked by inhibiting the CRF receptor (CRF-R), PKA and ERK pathways. In male beta-arrestin1 knockout mice, stress increases ISF Aβ levels nearly identically to females. Our data suggest that stress causes sex-dependent increases on Aβ and that are mediated by CRF-R/beta-arrestin signaling. Determining the cellular pathways that differ between the sexes could identify risks of developing AD and lead to therapeutics to specifically modulate the stress response in AD, potentially that vary by sex.

IN UTERO STRESS EXPOSURE ADVERSELY IMPACTS OFFSPRING NEUROCHEMISTRY AND BEHAVIOR

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Keywords: behavior, microdialysis, serotonin, citalopram, stress

Mood and anxiety disorders are the most prevalent psychiatric disorders affecting more than 300 million people worldwide. Risk factors, including genetic makeup and environmental factors, such as physiological and psychosocial stress, contribute to individual likelihood of developing a mood or anxiety disorder. Moreover, many pregnant women experience stressful pregnancies, which may promote a psychiatric disorder and thereby early developmental trauma. Human and animal studies have suggested that serotonin signaling plays an important role in the manifestation of and vulnerability to stress-induced affective disorders. Timed-pregnant mice underwent chronic, unpredictable stress during the latter half of their pregnancy, using ethologically relevant stressors. Some of the animals receive citalopram concomitantly through their drinking water. Post birth, tissue serotonin levels at three developmentally relevant timepoints for serotonin system maturation were assayed in the offspring. A subset of the offspring aged into adulthood, where they were tested using behavioral assays, *i.e.,* the elevated plus maze, open field test, forced swim test, and novelty suppressed feeding test. The offspring of stressed mothers had higher serotonin tissue levels and protein concentrations in the forebrain at postnatal day seven compared to control animals. Male adult offspring display greater anxiety-like behavior and higher corticosterone concentrations than sex-matched control animals. These effects were rescued in male animals whose mothers were exposed to concomitant citalopram. Current experiments are investigating whether maternal stress alters serotonin signaling in the ventral hippocampus, a brain region important for anxiety responses, in male adult mice. Preliminary findings indicate that male adults exposed to *in utero* stress have increased basal serotonin concentrations in the ventral hippocampus. Given the detrimental effects that prolonged exposure to negative psychosocial factors can have on physiological and cognitive functions, investigating the molecular mechanisms and brain circuits of these factors will be crucial to elucidate new therapies to mitigate their effects.

KAPPA OPIOID RECEPTOR MODULATION OF SEROTONIN IN DYSPHORIA

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Keywords : Microdialysis, stress, *in situ hybridization*

Clinical studies and animal models suggest that serotonin signaling plays an important role in the manifestation of mood disorders. A common symptom of anxiety and mood disorders is dysphoria defined as a state of profoundly unpleasant feelings or unease. Human and animal studies have shown that kappa opioid receptor (KOR) agonists produce dysphoric mood states. Research has also shown that while KOR agonists have anxiogenic effects, antagonists are anxiolytic. Nonetheless, the mechanisms by which KOR antagonists reduce anxiety-related or dysphoric behavior remain unclear. Our research investigates kappa-opioid receptor modulation of serotonin and dopamine signaling as a mechanism for producing dysphoric mood states. Pharmacologic manipulations are used to determine the mechanisms and circuitry of KOR modulation of the serotonergic and dopaminergic systems. Using *in vivo* microdialysis, we found that local perfusion of a KOR agonist decreased extracellular dopamine *while* increasing extracellular serotonin in the ventral striatum (vSTR). However, systemic injection of a KOR agonist did not produce similar changes in serotonin. Since stress is a major risk factor for anxiety and mood disorders, we investigated whether adult offspring exposed to maternal stress had altered KOR activation. We found that mice born to stressed dams showed increased extracellular serotonin after systemic administration of a KOR agonist in the ventral hippocampus (vHPC). This effect is only seen with concomitant local perfusion of the SSRI, citalopram. *In situ* hybridization in the dorsal raphe nucleus showed that *Oprk1, Sert, vGlut3* and *vGat* mRNA are coexpressed, indicating a possible direct or indirect mechanism for KOR-modulation of serotonin transmission. The overall goals of this project are to obtain a clearer understanding of the kappa opioid system in aversion and interactions with monoamine systems as they pertain to the neurocircuitry of aversive states. By elucidating the biological circuits involved in aversive states, new avenues for novel therapeutic targets in the treatment of mood disorders specifically targeting dysphoric symptoms can be explored.

THE TYPE OF DRUG INFUSION TECHNIQUE CRITICALLY INFLUENCES THE DYNAMIC OF NOREPINEPHRINE RELEASE IN THE RAT BASOLATERAL AMYGDALA

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Keywords: norepinephrine; α2-adrenoceptor drugs; basolateral amygdala; in vivo microdialysis; way of administration

The role of norepinephrine (NE) in learning memory processes in the rat has been shown to be mediated at least in part by the activity of the α2-adrenoceptor system in the basolateral amygdala (BLA). In reference to the effects induced by local infusion of the selective α2-adrenoceptor agonist and antagonist into the BLA on learning performances, the present study investigated whether the behavioural effects induced by local manipulation of the α2-adrenoceptor system are associated with biologically relevant changes in NE release. Two groups of male Sprague-Dawley rats were unilaterally microinfused or retrodialysed with in the BLA with the α2-adrenoceptor antagonist (idazoxan, 0.1 mM) or agonist (UK 14,304, 10 µM). Two other groups of animals received an i.p. infusion of one of the drugs (0.63 mg/kg). All groups were microdialysed in order to measure the effect of the drugs on NE release in the BLA. Dialysates were collected every 15 minutes for analysis of NE. The major finding of our study is that the type of administration of α2-adrenergic drugs greatly influences the level of NE release in the BLA. While NE release with i.p. and retrodialysis infusion of dexefaroxan and UK 14,304 reached a level similar to those described in the literature, however local microinfusion of both induced a release of NE that was 400% higher than baseline and local microinfusion of the agonist reduced NE levels during 90 min, that is much longer than this found in the literature. In conclusion, the present results strongly question the physiological relevance of the doses of α2-adrenoceptor drugs used with local microinfusion technique in behavioural experiments. In particular, due to the impact of such exaggerated release of NE on the α1- and b-adrenoceptor systems downstream activation, the interpretation of behavioural data obtained with this technique in terms of biological neural circuit underlying learning and memory processes should be revised.

TRIAZOLE1.1 AS AN EFFECTIVE ANALGESIC WITHOUT DOPAMINE INHIBITION IN AN ACUTE PAIN MODEL: ROLE OF BIOLOGICAL SEX

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Keywords : Dopamine, Microdialysis, Pain, KOR, Biological Sex

There is tremendous need for effective pain treatment without abuse liability. Pain induces a dysphoric state, in part though inhibition of dopamine (DA) release via activation of kappa opioid receptors (KORs), which have analgesic properties. Triazole 1.1 (Tri1.1) is a G-protein-biased KOR agonist which we theorize to have analgesic properties without DA inhibition, thus reducing the dysphoric aspects of pain. We tested this hypothesis by examining Tri1.1 alone (30 mg/kg s.c.) and with ketoprofen (10 mg/kg, i.p.) against pain-induced DA depression using a lactic acid pain model (0.54% lactic acid, i.p.), microdialysis, and HPLC detection of DA. We measured extracellular DA levels in male and female mice with microdialysis probes implanted in the nucleus accumbens (NAc). We found no effect of Tri1.1 alone on extracellular DA levels in male or female mice. We then demonstrated that lactic acid induced a decrease in DA levels in both male and female mice. We were able to prevent this decrease in both sexes by administering ketoprofen prior to lactic acid injection. Next, we looked at locomotor behavior as a proxy for pain, and found that lactic acid causes a significant decrease in the total distance traveled when compared to a control saline injection. This decrease was prevented in males but not females when Tri1.1 alone or Tri1.1 with ketoprofen was given prior to the lactic acid injection. Our results showed no sex differences in extracellular DA responses to Tri1.1 (no change) or lactic acid (decreased DA), or the efficacy of ketoprofen in preventing lactic acid-induced DA reduction. However, Tri1.1 was only able to prevent lactic acid-mediated hypo-locomotion in males. Taken together, these data suggest sex-dependent pain responses, such that Tri1.1 and Tri1.1 with ketoprofen ameliorate pain-related behavior in males, but are not sufficient to ameliorate pain behavior in females. Additionally our microdialysis data indicates that Tri1.1 does not reduce DA levels in either males or females, unlike full KOR agonists. These findings support the use of Tri1.1 as an analgesic in males, especially in combination with traditional pain treatments.

UNCOVERING THE RELEASE DYNAMICS OF ENKEPHALINS FOLLOWING ACUTE STRESS

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Keywords : Neuropeptides, Microdialysis, Liquid Chromatography/Mass Spectrometry, Stress, Opioids

Opioid peptides are key modulators of natural reward and threat processing. It has been shown in the rodent models that enkephalinergic neurons in the nucleus accumbens (NAc) shell may play a modulatory role in stressful situations, for example after the exposure to predator odor and in the vulnerability to social defeat stress. Here, we show that enkephalinergic neurons in the ventral NAc shell are activated following exposure to predator odor and experimenter handling using fiber photometry. To get a deeper insight into enkephalin release dynamics we couple microdialysis and nano-liquid chromatography/mass spectrometry to allow the detection of endogenous Leu- and Met-enkephalin *in vivo*. With this approach we can detect and distinguish between Met- and Leu- Enkephalin release in awake behaving mice at sub femtomolar concentrations. We show that enkephalins are released following exposure to predator or handling stress. We also demonstrate the dynamics of Met and Leu Enkephalin release as well as how they correlate to one another in the ventral NAc shell. This detection method allows for the real-time quantification of Met and Leu enkephalin release in the ventral NAc shell following acute stressors. In the future, we plan to use this technique in different behavioral contexts and hope to translate it to human samples.

DEFECTS IN MOUSE CORTICAL GLUTAMATE UPTAKE CAN BE UNVEILED IN VIVO BY A TWO-IN-ONE QUANTITATIVE MICRODIALYSIS

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Keywords: glutamate uptake; in vivo; motor cortex; quantitative microdialysis; reverse dialysis

In the brain, extracellular glutamate (Glu) levels are maintained low by efficient transporters, whose dysfunction is implicated in neuronal hyperexcitability, excitotoxicity, and many neurological diseases. While many methods can estimate Glu uptake *in vitro* or *ex vivo*, a limited number of techniques addresses Glu transport *in vivo*. Here, we used in vivo microdialysis in a two-in-one approach combining reverse dialysis of isotopic Glu to measure uptake ability and zero-flow (ZF) quantitative microdialysis to sample and quantify extracellular Glu levels. We applied the microdialysis protocols to wild-type mice and DMSXL mice, a transgenic model of human myotonic dystrophy type 1 (DM1) neuromuscular disease, characterized by low levels of the EAAT-2/GLT1 glutamate transporter, among other molecular and functional brain defects. DMSXL mice displayed a ~20% reduction in GLT1 levels in the cortex, and our microdialysis protocol was sensitive enough to unveil *in vivo* a decrease in cortical Glu uptake. Additionally, on the same animals, we found abnormal extracellular Glu levels determined by ZF method in line with GLT1 defects. However, the “plateau” profile of Glu ZF data was different from that usually obtained with the theoretical ZF model. Three mathematical models were used to fit the shape of ZF Glu data in both wild-type and DMSXL mice. The use of mathematical fitting on the observed “plateau” shape revealed a striking positive correlation between Glu uptake and Glu levels in DM1 mouse strain. In conclusion, we propose a sensitive two-in-one microdialysis approach that is robust enough to reveal significant differences in neurotransmitter uptake and extracellular levels through the analysis of a relatively low number of animals (n≤6 per group).

DM1 TRANSGENIC MICE EXHIBIT ABNORMAL NEUROTRANSMITTER HOMEOSTASIS AND SYNAPTIC PLASTICITY IN ASSOCIATION WITH RNA MIS-SPLICING IN THE HIPPOCAMPUS

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Keywords: GABA; RNA splicing; glutamate uptake; synaptic plasticity; transgenic mouse model

Myotonic dystrophy type 1 (DM1) is a severe neuromuscular disease mediated by a toxic gain of function of mutant RNAs. The neuropsychological manifestations affect multiple domains of cognition and behaviour, but their aetiology remains elusive. Transgenic DMSXL mice carry the human DM1 mutation and show relevant behavioural abnormalities (including altered exploratory behaviour and anhedonia) and express reduced levels of GLT1, a critical regulator of glutamate homeostasis in the brain. However, the impact of glutamate homeostasis dysfunction on neurotransmission in DM1 remains unknown. In the present study, we show an *in vivo* reduced glutamate uptake in the DMSXL hippocampus compared to wildtype mice, but extracellular glutamate levels sampled in the dorsal hippocampus using zero-flow quantitative microdialysis were unaltered. Patch clamp recordings of CA1 pyramidal neurons and DG granule cells in hippocampal slices from DMSXL mice revealed an increased tonic excitation, likely mediated by higher levels of ambient glutamate at the vicinity of extrasynaptic NMDA receptors. We also found an unexpected elevated extracellular GABA level in DMSXL mice associated with an increase in tonic inhibition and a higher GABA release. Finally, we found evidence of abnormal short-term plasticity in the DG and CA1 area, suggestive of synaptic dysfunction in DMSXL mice. Synaptic dysfunction was accompanied by the accumulation of RNA foci that are more abundant and larger in the DG than in CA1 area, and by the mis-splicing of candidate genes with relevant functions in amino acidergic neurotransmission (ion channels, neurotransmitter receptors and synaptic proteins, as well as proteins involved in neuronal vesicle trafficking). Taken together, molecular and functional changes triggered by the accumulation of toxic RNA may induce synaptic abnormalities in restricted brain areas of the brain, causing neuronal dysfunction.

OPTOGENETIC STIMULATION OF MIDBRAIN DOPAMINE NEURONS INDUCES STRIATAL SEROTONIN RELEASE

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Keywords : Microdialysis, striatum, optogenetics, serotonin, Chrimson

Targeting neurons with light-driven opsins is widely used to investigate cell-specific responses. Here, we aimed to determine the magnitude of extracellular dopamine release in the dorsal striatum (dSTR) upon optogenetic stimulation of midbrain dopaminergic neurons. Dopamine neurons were optogenetically stimulated in the ventral tegmental area (VTA)/ substantia nigra (SN) in mice selectively expressing the excitatory opsin, Chrimson, under the control of the dopamine transporter promoter. Changes in extracellular dopamine levels in the dorsal striatum were determined by fast microdialysis using electrochemical detection in awake mice to investigate the extent of dopamine release in response to optogenetic stimulation. Since we separate and detect dopamine and serotonin in 5-min online sampling, we investigated basal and stimulated levels of both neurotransmitters. Optical stimulation of dopamine neurons resulted in increases in dopamine, serotonin, and 3-methoxytyramine (3-MT), a dopamine metabolite. To investigate the neuronal circuitry underlying corelease of dopamine and serotonin, D1 and D2 receptor antagonists were perfused through the microdialysis probe into the dorsal striatum. Local application of the D1 antagonist SCH 23390 was associated with elevated basal dopamine and serotonin levels. The D2 antagonist eticlopride potentiated optically evoked dopamine release. Neither antagonist prevented optically stimulated increases in striatal serotonin levels. These results, in addition to our findings using rapid pulse voltammetry to investigate optically stimulated dopamine and serotonin, suggest that striatal dopamine receptors do not mediate co-release. Other possibilities, which we are investigating, include serotonin release from dopaminergic vesicles (false transmitter), dopaminergic activation of dorsal raphe serotonin cell bodies, or the involvement of D1 or D2 receptors outside of the striatum. In any case, these findings highlight functional connectivity of neurotransmitter systems that should be considered when interpreting optogenetic control of behavior.

Optogenetic stimulation of midbrain dopamine neurons produces striatal serotonin release. ACS Chemical Neuroscience. (2022), DOI: 10.1021/acschemneuro.1c00715.

PERIPHERAL MACROPHAGE D2 RECEPTORS MEDIATE ETHANOL ENHANCEMENT OF DOPAMINE TRANSMISSION IN THE NUCLEUS ACCUMBENS

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Keywords : Ethanol, dopamine, D2 receptors, neuroimmune, macrophage

The rewarding properties of ethanol (EtOH) appear to be mediated by enhancement of dopamine (DA) transmission in the mesolimbic DA system originating in the ventral tegmental area (VTA) of the midbrain and projecting to the nucleus accumbens (NAc) of the striatum. Ethanol is known to both enhance DA neuron firing in the VTA and DA levels in the NAc, which have been implicated in reward learning. The aim of this study was to investigate the effects of peripheral DA 2-subtype receptor (D2R) antagonism on EtOH effects on DA transmission and to determine the role of peripheral D2R-expressing macrophages and microglia in mediating EtOH effects.

Using voltammetry and microdialysis, we evaluated EtOH effects on DA release in the NAc of Wistar rats and MaFia mice that express a GFP reporter and suicide transgene for myeloid-derived macrophages. A place conditioning paradigm was used to assess conditioned preference (CPP) for EtOH and whether administration of the peripheral-only D2R antagonist domperidone (DOM) altered this preference. Open field and loss of righting reflex paradigms were used to assess the effects of DOM on EtOH locomotor activity and sedation. A rotarod apparatus was used to assess the effects of DOM on EtOH-induced motor impairment. Using single-unit electrophysiology we evaluated the effects of the macrophage inhibitor clodronate on single-unit activity in the VTA and EtOH sedation. Using fluorescence assisted flow cytometry we evaluated the infiltration of D2R expressing macrophages and numbers of microglia.

Domperidone (DOM) attenuated both intraperitoneal EtOH and intravenous DA enhancement of DA release in the NAc. Domperidone also decreased EtOH-induced sedation at 2.0 g/kg. Domperidone did not alter EtOH CPP, nor did it affect EtOH-induced motor impairment. Using volume/surface area measurements, microglia in the VTA and NAc shifted toward an M1 inflammatory phenotype with acute intoxicating levels of EtOH, in particular 2 g/kg. Although EtOH increased the expression of D2Rs in microglia, it decreased the number of infiltrating monocytes in the VTA and NAc. Administration of clodronate liposomes depleted macrophages in the plasma 50% and blocked EtOH inhibition of VTA GABA neuron firing rate and locomotor activity.

These results demonstrate that peripheral D2Rs on macrophages mediate some of the effects of EtOH on mesolimbic DA neurotransmission, although these effects are not specifically related to the rewarding properties of EtOH.

3D PRINTING MICROSAMPLING PROBES

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Keywords : 3D printing, microdialysis, sampling devices

For more than 40 years, microdialysis (μD) sampling has been used in neuroscience applications to collect low molecular weight solutes, peptides, and proteins. In this timeframe, few alterations to the currently used and commercially available cylindrical μD sampling probes have been described; the exception being the use of established photolithography techniques to fabricate smaller sized μD probes with improved spatial resolution. The challenge with these standard processes is the tremendous challenge to include complex microfluidic geometries, such as the herringbone passive mixing design. To overcome these limitations, 3D printing has become a widely used manufacturing technique for incorporating geometric features in the 100-μm range. Yet, for microdialysis sampling, this is still too large of a feature size. To get the necessary feature sizes for microdialysis sampling, a combination of two 3D printing technologies have been paired to make an original prototype to use initially for push-pull sampling. The first uses the Nanoscribe GT two-photon stereolithography 3D printer, allowing fabrication of complex geometries with 200 nm feature sizes. The Nanoscribe (NS) is used to print a 4 mm long subsection that includes the sampling needle consisting of a 200 μm triangular cross-section. This needle contains an array of 3330 x 5 μm pores that comprise a fully 3D-printed membrane. The NS-printed needle section is then combined with a separately 3D printed inlet/outlet and structural support section that is printed in less than 30 minutes on an Anycubic Mono 4K masked-stereolithography 3D printer, which is capable of 34 μm feature sizes. The two 3D printers are necessary to make the device as the print times for the Nanoscribe increase quadratically with feature size reductions. The Nanoscribe needle section consists of only 1.16% of the total device volume and is completed in 3 hours. The dual printer method allows for the rapid fabrication, characterization, and revision of highly customized and complex microsampling probe designs that are otherwise impossible to create individually. We acknowledge the Arkansas Biosciences Institute for funding.

INTRACRANIAL ELECTROPHYSIOLOGICAL BIOMARKERS OF COMPULSIVITY IN OBSESSIVE-COMPULSIVE DISORDER

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Keywords: Obsessive-compulsive disorder (OCD), biomarker, local field potential (LFP), deep-brain stimulation (DBS), basal ganglia

Compulsivity is a hallmark of many psychiatric disorders and the nuclear symptom of obsessive-compulsive disorder (OCD). A dysfunction of neuronal network activity in cortico-striatal circuits is hypothesized to underlie compulsivity, however, its exact physiological manifestation is yet to be discovered. To improve clinical outcomes, it is essential to identify biomarkers of compulsivity, so they may be specifically targeted with deep-brain stimulation (DBS). To this end, electrophysiological data recorded from implanted DBS electrodes in OCD patients may provide invaluable insight into specific pathological neuronal activity we may treat.

We present local-field potential (LFP) data recorded in 11 OCD patients throughout symptom provocation, with the aim to identify neural signatures of compulsivity. LFP was recorded using the Medtronic PC+S DBS system, from leads implanted throughout basal ganglia and associated white matter structures. Recording sites were identified by localizing the electrodes using Lead-DBS. Spectral analysis of LFP data was done using Fieldtrip. Spectral estimates were tested for significance using a nonparametric randomization test, corrected for multiple comparisons, for each brain region across frequency bins.

We identify electrophysiological correlates of compulsive behavior throughout the basal ganglia across patients. Low-frequency LFP power is increased during compulsions in external globus pallidus (GPe), nucleus accumbens, anterior limb of the internal capsule, and anterior commissure. In GPe, this compulsivity-signature is independent from compulsive action, and distinct from obsessions and relief; the signal is more pronounced in the anterior tip of GPe than more posterior recording locations.

We observe LFP power spectrum changes during compulsions throughout basal ganglia. Predominantly in GPe, this altered network activity may be involved in the generation or execution of this behaviour. Thus, the basal-ganglia electrophysiological biomarkers of compulsivity identified in this study pave the way toward development of biomarker-targeted closed-loop DBS for OCD.

IMPACT OF HIPPOCAMPAL UPREGULATION OF ADENOSINE A_{2A} RECEPTORS IN THE MOUSE HIPPOCAMPUS

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Keywords : Adenosine A_{2A} receptor, Astrocyte, Hippocampus, Memory

Alzheimer's disease (AD) is characterized by the intraneuronal aggregation of tau proteins which leads to synaptic dysfunctions and memory decline. Studies have reported that chronic caffeine consumption reduces AD risk and cognitive deficits. These protective effects would be ascribed to the blockade of adenosine A_{2A} receptors (A_{2A}Rs) which are pathologically upregulated in hippocampal astrocytes of AD patients and whose levels have been correlated with the development of cognitive deficits. However, the mechanisms underlying the link between astrocytic A_{2A} upregulation and cognitive deficits remain unknown.
To uncover the effects of astrocytic A_{2A}R upregulation, we intrahippocampally injected an AAV2/9 virus allowing A_{2A}R expression (AAV-A2A), or GFP as control (AAV-GFP), under a GFAabc1d astrocyte-specific promoter, in 2m-old C57Bl6/J mice. We then evaluated impact of astrocytic A_{2A}R upregulation on spatial memory performances, response to hippocampal neural networks using DREADD technology as well as astrocyte reactivity, morphology and transcriptome.
Our data show that A_{2A}R overexpression in hippocampal astrocytes impairs short-term spatial memory and long-term spatial learning. At the network level, thanks to a DREADD approach, we observed an enhanced neuronal activability of hippocampal neurons following A_{2A}R astrocytic overexpression. These neuronal changes were associated with changes in astrocyte reactivity, 3D morphology and transcriptome.
These results demonstrate that A_{2A}R upregulation in hippocampal astrocytes, as seen in the brain of AD patients, is sufficient to alter the astrocytic phenotype, neuronal response and memory. Further experiments in AD mouse models will determine how this A_{2A}R deregulation in astrocytes potentiates the development of AD lesions and their consequences, but also whether astrocytic A_{2A}R downregulation is sufficient to bring benefits.
Our projects are supported by ANR ADORASTrAU, Fondation Alzheimer (ADOMEMOTAU) and LabEx DISTALZ. AL is supported by a doctoral grant of FRM (ECO202106013670).

CHEMICAL BIOPSY AS A NEW TOOL IN PROFILING OF THE HUMAN BRAIN IN VIVO

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Keywords : chemical biopsy, SPME, lipidomic, brain tumor, on-site

Biochemical analysis of brain tissue was always challenging because of the limited access to this biological material. Thus, a low invasive tool that allows obtaining analytes for neuroscientific or diagnostic purposes is highly desired. The study presented herein aimed to assess the applicability of solid-phase microextraction (SPME) for in vivo characterization of white and grey matter in human brain immediately before the biopsy as well as for the differentiation of non-neoplastic tissue from the tumor. Sampling was conducted during brain tumor biopsy procedures. SPME probes were introduced for 4 minutes simultaneously into white and grey matter not affected by neoplastic changes. Then, the sampling of brain tumors was performed directly after lesion removal. After collecting all the samples metabolomic and lipidomic analyses were performed with the use of the LC-HRMS platform. The results of this study showed that a wide range of small metabolites was extracted from sampled tissue. The majority of them were lipids, such as triglycerides (TG), phosphatidylcholines (PC), phosphatidylethanolamines (PE). Relatively weak separation of white and grey matter was observed. However, the comparison of lipid profiles obtained from healthy tissue and neoplastic lesions were significantly altered. The present study, considered a proof-of-concept experiment, was the first attempt of in vivo characterization of the metabolite profile of white and grey matter in the human brain as well as of a comparison of the metabolomic profile of healthy and neoplastic tissue. However, extended studies should be continued on a larger cohort to draw solid conclusions about the biochemistry of the live human brain. The work was supported by the grant provided by the Ministry of National Defence of Poland under the second edition of the Kościuszko program (508/2017/DA). The authors would like to thank Supelco Inc. (Merck) for supplying the SPME probes

STRUCTURED TRACKING OF ALCOHOL REINFORCEMENT REVEALS DISTINCT CORTICAL AND BRAINSTEM BIOMARKERS OF COMPULSIVE DRINKING

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Keywords : AUD, Ethanol, LC-MS, mPFC, Operant

Alcohol is a widely used and abused drug that has been studied across diverse fields of research. While many studies focus on the pathophysiology of alcohol use disorder (AUD), the need for models that are valid at the basic and translational level has become increasingly apparent. Here, we develop a novel modular behavioral paradigm: Structured Tracking of Alcohol Reinforcement (STAR). STAR provides a robust framework for quantitative assessment of AUD-related behaviors while still allowing flexibility in overall experimental design. We use the STAR framework to define longitudinal phenotype dynamics in AUD-relevant behaviors over the course of repeated alcohol use and binge drinking. However, the adaptable design allows for any number of other methodologies, investigative or intervening, to be easily integrated to tailor for the specific question being asked. Further, using STAR in combination with tissue analytics, we reveal putative neuro-biomarkers of heightened alcohol abuse. Tissue from medial prefrontal cortex (mPFC) and dorsal periaqueductal gray area (dPAG) was neurochemically screened through LC-MS to investigate biomarkers across the drinking phenotypes that were implicated in AUD-related behaviors. These results revealed multiple analytes in both regions that differed significantly across phenotypes. For example, dopamine- and 5-HT-related analytes in both the mPFC and dPAG were positively correlated with ethanol intake, but not during punished sessions, whereas excitatory and inhibitory transmitters in the dPAG were positivity correlated with punishment resistance. Overall, our results provide a novel and robust platform for studying the development of drinking behavior in mice that is both flexible and easily integrated with existing technologies, making it a strong model for studying the development of increased alcohol use and compulsion from a behavioral, circuit, and population perspective. Lastly, our data points to several neural analytes in cortical and brainstem regions that imply the possibility of underlying neuro-biomarkers for vulnerability to compulsive drinking behaviors.

EFFECTS OF THE 5-HT2A RECEPTOR AGONIST TCB-2 ON THE NEUROCHEMISTRY OF MONOAMINES AND AMINO ACID NEUROTRANSMITTERS IN THE MOUSE STRIATUM

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Keywords: psychedelics, neurochemistry, correlative analysis, basal ganglia, MDL100907

TCB-2 is a preferential 5-HT2A receptor (5-HT2AR) agonist that induces classical signs of serotonergic hallucinogenic drugs in rodents. These drugs modulate neurotransmitter contents in various brain regions, but it is unclear if the responses are homogenous inside a brain region. The striatum has distinct functional territories allowing us to address 1) the neurochemical effects of TCB-2 within the ventromedial, ventrolateral, dorsolateral, and dorsomedial striatum, and 2) the connection of the neurotransmitter content between at least two of these regions (ventromedial, ventrolateral) using correlative analyses. Tissue levels of monoamines [dopamine (DA), noradrenaline (NA), serotonin (5-HT)], their metabolites, and amino acids neurotransmitters (aspartate, glutamate, glycine, GABA) were measured using HPLC coupled to electrochemical detection in the striatum of male C57Bl6/J mice one hour after the intraperitoneal injection of TCB-2 (0.3, 3, 10 mg/kg). A behavioural observation indicated that mice receiving TCB-2 at 3, 10 but not 0.3 mg/kg displayed head-twitched response, an effect blocked by the selective 5-HT2AR antagonist MDL100907 (0.2 mg/kg, 15 minutes before 3 mg/kg TCB-2). TCB-2 did not alter the striatal levels of amino acid neurotransmitters, NA, tended to enhance DA (mainly ventrolateral), and increased 5-HT content at the highest dose. TCB-2 dose-dependently and robustly reduced the 5-hydroxyindoleacetic acid/5-HT and 3-methoxytyramine/DA ratios in some striatal regions. The 5-HT2AR antagonist MDL100907 counteracted the effect of TCB-2 (3 mg/kg) on 3-methoxytyramine/DA ratio but not on the 5-HIAA/5-HT ratio. The strong correlative link reported for the neurotransmitter contents between the ventrolateral and the ventromedial striatum in control animals was reduced or suppressed in TCB-2-treated mice. The analysis of the data is still ongoing to include all striatal quadrants. Based on our preliminary findings, TCB-2 homogeneously inhibits in the two ventral striatum the DA and 5-HT metabolisms involving or not 5-HT2ARs, respectively, and disrupts the correlative links for all neurotransmitter contents between the two striatum.

ORCHESTRATING THE BRAIN MONITORING OF MONOAMINE METABOLISMS AT REST IN THE IMPULSIVITY/COMPULSIVE DIMENSIONS IN RATS

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Keywords: impulsivity, monoamines, inter-individuality, neurochemistry, correlative analyses.

This study aims to investigate the patterns of dopamine (DA) and serotonin (5-HT) metabolisms at rest within selected brain regions related to inter-individual variability in six main components of impulsivity/compulsivity (anticipatory hyperactivity, premature responses, delay discounting, risk taking, perseverations and flexibility).
35 naive rats were screened for their behavioural responses in five distinct tests addressing six components of impulsivity/ compulsivity: the Fixed Consecutive Number of 16 lever press schedule (premature responses), the multiple Fixed-Interval/ Extinction schedules of reinforcement (anticipatory hyperactivity and perseveration), the Delay Discounting Task (impulsive choice), the Rat Gambling Task (behavioural flexibility) and the light-dark emergence test (risk taking). They were divided by extracting subgroups of individuals with low or high scores according to the upper and the lower terciles in each task (n=12 ± 2). After the completion of the behavioural experiment, the content of monoamines and metabolites was monitored using high pressure liquid chromatography coupled to electrochemical detection in 20 brain areas (10 cortical and 10 subcortical areas).
Distinct patterns of 5-HT and DA metabolisms were revealed according to the behavioural traits. Except for hyperactive responses, lower control of actions was mainly associated with a lower DA or 5-HT metabolism in prefrontal and/or subcortical areas (i.e. in orbitofrontal cortex (DA), amygdala and anterior cingulate cortex (5-HT) for inflexible and risk prone rats). Correlative analysis (connectivity) confirmed distinct monoaminergic mapping between well adapted and maladaptive responders in each test. Notably lower DA and 5-HT metabolisms were found in dorsolateral striatum of risk taking rats, an effect associated with a lack of correlative link with the ones of other brain regions.
In conclusion, specific behavioural traits related to impulse control disorders are associated with distinct monoaminergic function at rest. The changes of monoamines are quantitatively brain region specific and are associated with broader changes of connectivity.

A NOVEL WIRELESS ELECTROCHEMICAL DEVICE FOR REAL-TIME MONITORING OF NEURAL IONIC CHANGES IN PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY

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Keywords: Traumatic Brain Injury, Spreading Depolarisation, Microdialysis, CMOS Technology, Ionophore.

Worldwide, traumatic brain injury (TBI) affects almost 69 million people per year, of which 10-15% of cases are severe, requiring careful clinical care in an intensive therapy unit (ITU) [1]. TBI is an evolving injury that can worsen over time and result into an exacerbation of the neurologic damage, known as secondary brain injury (SBI), which increases the risk of poor outcomes. A continuous and real time monitoring can reveal the underlying mechanisms of the injury and offer medical support to find the best neuroprotective strategy for preventing patients' deterioration.

We present a wireless bedside monitoring device for the detection of ionic changes in cerebral microdialysate streams to identify spreading depolarisation (SD) events, which are disruptive cortical depolarisation waves considered a hallmark of SBIs [2]. We employ an electrochemical device based on Complementary Metal-Oxide-Semiconductor (CMOS) technology that consists of a 3X2mm array of 4756 Ion Sensitive Field Effect Transistors (ISFETs). The sensing surface is coated with ionophore membranes specific to potassium, sodium, and calcium, whose concentration is substantially changed following a SD event [3]. A 3D printed microfluidic flow-cell allows the manipulation of small volumes in the order of uL, which makes the device suitable for the analysis of dialysate streams that are very limited in volume. The flow cell communicates with a programmable microfluidic board, that enables an automated calibration of the device prior the measurement [4]. The recordings are continuously processed and shown in a user friendly and dedicated app. According to preliminary results, the device exhibits a good multi-ion sensitivity with a decent time response which makes its future implementation in ITU settings for a fast detection of SDs very promising.

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INFLUENCE OF NEUROMODULATORY SYSTEMS IN THE HINDLIMB REPRESENTATION IN THE DEVELOPING SOMATOSENSORY CORTEX OF THE RAT

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Keywords: Serotonin (5-HT), Choline Acetyl Transferase (ChAT), Tyrosine Hydroxylase (TH), synaptic transmission, volume transmission

The vast majority of cortical synapses are found in the neuropil which is implicated in multiple and diverse functions underlying brain computation. Unraveling the organizing principles of the cortical neuropil requires an intricate characterization of synaptic connections established by excitatory and inhibitory axon terminals, of intrinsic and extrinsic origin and from ascending projections that govern the function of cortical microcircuits through the release of neuromodulators either through point-to-point chemical synapses or diffuse volume transmission. The hindlimb representation of the somatosensory cortex (HLS1) of two-week old Wistar rats has served as a model system to dissect the microcircuitry of neurons and their synaptic connections. In the present study, we quantified the fiber length per cortical volume and the density of varicosities for catecholaminergic, serotonergic and cholinergic neuromodulatory systems in the cortical neuropil using immunocytochemical staining and stereological techniques. Acquired data were integrated into a computational modeling framework to reconcile the specific modalities and predict the effects of neuromodulatory release in shaping neocortical network activity. We predict that ACh and DA and 5-HT desynchronize cortical activity by inhibiting slow oscillations (delta range), and that 5-HT triggers faster oscillations (theta). Moreover, we found that high levels (>40%) of neuromodulatory volumetric transmission (VT) are sufficient to induce network desynchronization, but also that combining volume release with synaptic inputs leads to more robust and stable effects, and lower levels of VT are needed to achieve the same outcome (10%).

CORTICOSTRIATAL DYSFUNCTION IN SAPAP3 KNOCKOUT MICE GOVERNS COMPULSIVE BEHAVIOR

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Keywords : Schedule-induced polydipsia, Sapap3-KO mice, corticostriatal circuits, compulsive behavior, grooming

Compulsivity, a core symptom of obsessive-compulsive disorder (OCD), consists of the performance of repetitive behavior, driven by an urge that persists despite adverse consequences. Although a dysfunction of corticostriatal circuits is thought to underlie such compulsive behavior, much more knowledge is required to improve clinical outcomes of OCD treatment. The genetic deletion of the Sapap3 protein in mice induces corticostriatal dysfunction and excessive grooming. Using Sapap3 knockout mice (Sapap3-KO) and wild-type controls, we evaluate the effects of this genetically-induced corticostriatal dysfunction as well as the effects of targeted impairment of the orbitofrontal cortex (OFC) on two forms of compulsive behavior: excessive water drinking in the schedule-induced polydipsia (SIP) paradigm, and “spontaneous” compulsive grooming.
Compulsive behavior is induced by daily exposure to the SIP paradigm: food-restricted mice are presented with intermittent food-pellet delivery in an operant box, while given free access to a water bottle. Under these conditions, some mice develop excessive water drinking persistent across weeks, while others remain “low drinkers”. We compared acquisition of compulsive drinking in SIP between Sapap3-KO and wild-type mice that were given either excitotoxic lesions of the OFC by intracranial injection of quinolinic acid prior to SIP training, or sham controls. Additionally, we compared grooming behavior before and after OFC lesion and before and after SIP training.
Preliminary results indicate that both Sapap3-KO and wild-type mice may develop excessive drinking in the context of SIP. Additionally, OFC lesions increase water intake in Sapap3-KO mice, but not in wild-type mice, compared with sham controls. In turn, OFC lesions did not affect grooming in Sapap3-KO or wild-type mice.
We find that compulsive drinking in the Sapap3-KO mouse model is exacerbated by OFC lesion, suggesting that the OFC plays a role in preventing the development of compulsive behavior. This identifies the OFC as a relevant target for future studies characterizing its role in compulsivity.

COATED BLADE SPRAY-MASS SPECTROMETRY AS A NEW APPROACH FOR QUANTITATIVE ANALYSIS OF GLIOMAS

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Keywords : SPME; CBS-MS; brain tumors; gliomas; lipidomics

Gliomas are brain tumors with one of the highest mortality rates, which is related mostly to their wide histological and molecular diversity. Therefore, the critical importance for patient outcomes is the accessibility of methods that allow for the quick and effective diagnosis of this neoplasms, and hence the selection of appropriate treatments.
The purpose of the presented study was to test the application of coated blade spray-mass spectrometry (CBS-MS) method in targeted analysis of gliomas.
The analysis was conducted on one glioma tumor using CBS-MS, the method which combines the features of microextraction and fast ionization methods. The sword shape device, coated with a sorptive material, was inserted into the studied tissue to extract analytes, in this case carnitine. Then, the probe was installed into interface on the mass spectrometer source and extracted analytes were desorbed using 20ul of desorption solvent. Subsaquently, after 45s high voltage was applied to ionize and analyze the level of carnitine.
The concentration of carnitine in the homogenate was calculated based on the standard addition method. Obtained results revealed that the order of magnitude of carnitine concentration was in concordance with the literature reports. Apart from homogenate analysis, CBS probes were also used in the intact tissue sampling. It should be noted that the highest intensity of the carnitine peak was observed in the homogenate sample, while the values obtained for the intact tissue were generally lower. Nevertheless, the relative concentration obtained from the intact tissue still corresponded to the concentration measured in the homogenate calculated based on the standard addition method.
To sum up, presented experiment shows that CBS-MS technology could be useful tool in quantitative analysis of endogenous analytes. However, extended studies on CBS-MS method optimization, as well as analysis of a larger cohort of patients should be conducted.
This research was funded by the National Science Center Poland within research grant no. 2015/18/M/ST4/00059 entitled “New analytical solutions in oncology: From basic research to rapid intraoperative diagnostics”.

SUB-SECOND FLUCTUATIONS OF EXTRACELLULAR DOPAMINE IN HUMANS ENCODE *VALENCE -PARTITIONED* REWARD AND PUNISHMENT PREDICTION ERRORS

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Keywords: human voltammetry, dopamine, prediction error, reinforcement learning, subjective experience

The rate at which action potentials are generated at the soma of dopamine releasing neurons has been shown to encode temporal difference (TD) reward prediction errors (RPEs). Downstream, the release of dopamine into the extracellular space is not guaranteed to reflect this calculation. Data from non-human and human experiments suggest that fluctuations in extracellular dopamine levels on sub-second timescales is more complex than the long-standing TD-RPE hypothesis about dopamine neuron activity – including data that support the idea that dopamine release may occur to aversive or salient stimuli. Here, we test the hypothesis that dopamine release in human striatum encodes TD-RPE using fast scan cyclic voltammetry performed during brain surgery. Patients with essential tremor undergo brain surgery to implant deep brain stimulating electrodes to manage chronic debilitating motor symptoms. These patients are awake during the procedure and, by all accounts, have an intact dopaminergic system. This permits an opportunity to measure and investigate the role of sub-second changes in extracellular dopamine levels in awake humans while they perform experimental tasks. We demonstrate that dopamine release does encode TD-RPEs when outcomes are rewarding (or expected to be rewarding), but not when outcomes are (or expected to be) punishing. We propose a *valence-partitioned* model for efficient learning of appetitive and aversive events and show that this model is a better explanation of dopamine release in these experiments. Notably, our new approach retains optimal learning rules from TD-reinforcement learning theory but extends the algorithm to allow independent encoding and learning of aversive experience. This approach explicitly accounts for the possibility that different sub-populations of (dopamine or other neuromodulatory) neurons may encode rewarding versus punishing events. Further, this model allows for a parsimonious explanation regarding the relationship between prediction error signaling at the soma and aversive or saliency encoding at release sites throughout the mammalian brain.

IMPACT OF POSTNATAL TTX BLOCKADE OF THE VENTRAL SUBICULUM ON MK-801 INDUCED DOPAMINERGIC RESPONSES IN THE NUCLEUX ACCUMBENS

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Keywords : Animal modelling; schizophrenia; ventral subiculum neonatal inactivation; nucleus accumbens; *in vivo* voltammetry

The present study, carried out in the context of the animal modelling of schizophrenia, aimed to investigate in adult rat the consequences of a transient neonatal functional inactivation of the ventral subiculum (SubV) on locomotor activity and dopaminergic (DAergic) responses in the dorsomedian *shell* part of the nucleus accumbens (Nacc) after subcutaneous (s.c.) injection of a very specific non-competitive antagonist of NMDA receptors: the MK-801. The functional neonatal inactivation of SubV was achieved by local microinjection of tetrodotoxin (TTX) at postnatal day 8 (PND8); control pups were microinjected with the solvent phosphate buffered saline (PBS). Locomotor responses and DAergic variations in the *shell* part of the Nacc were measured using *in vivo* voltammetry in awake, freely moving animals after administration of NaCl 0.9% ; MK-801 0.1 mg/kg or MK -801 0.2 mg/kg. The following results were obtained: 1) a dose-dependent increase in locomotor activity in PBS and TTX animals, greater in TTX rats/PBS rats ; 2) divergent DAergic responses for PBS and TTX animals. A decrease in DA levels with a return around basal values was observed in PBS animals. An increase in DA levels was obtained in TTX rats for both doses of MK-801. These data suggest that the neonatal functional inactivation of SubV by TTX at PND8 leads to dysregulation of DA release in the Nacc *shell* in adults rats, involving NMDA receptors. These results may contribute to the validation of the animal modelling of the pathophysiology of schizophrenia, consistent with the hypothesis of cerebral disconnections of neurodevelopmental origin.

LOCUS COERULEUS NORADRENERGIC PROJECTIONS TO THE MEDIAL PREFRONTAL CORTEX DRIVE AVOIDANCE BEHAVIORS

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Keywords : prefrontal cortex, avoidance, locus coeruleus, optogenetics, norepinephrine

The locus coeruleus noradrenergic system (LC-NE) broadly impacts physiological functions including arousal, memory, cognition, pain processing and stress reactivity. The LC-NE system has been implicated as a key mediator of acute stress-induced anxiety and aversive behaviors. We previously showed that high tonic LC-NE activity is required to elicit acute stress-induced anxiety, is aversive in real-time, and can affect future behavior through learned association. The medial prefrontal cortex (mPFC) receives extensive projections from LC and is involved in many of the same behaviors modulated by the LC-NE system. In this study we aimed to determine the role of the LC-NE projections to the mPFC in modulating aversive and approach-avoidance behaviors in mice. We used optogenetics to selectively target channelrhodopsin-2 (ChR2) to LC-NE neurons of *Dbh*-Cre mice and stimulated LC-mPFC noradrenergic projections with 5 Hz (10 ms pulse width) across a battery of behaviors. Our results indicate that concurrent stimulation of the LC-NE projections in the mPFC significantly reduces time spent in the open areas of the elevated plus maze. This decreased exploration also occurs when animals are stimulated prior to the open field test. Likewise, *Dbh*-Cre$^{LC\text{-}mPFC:ChR2}$ mice had a significant conditioned place aversion from the photostimulated context compared to Cre- controls. However, when mice were tested in the real-time preference test no difference was found between the two groups, suggesting that LC-mPFC stimulation itself may not be sufficient to immediately induce aversion. Preliminary data suggests that inhibition of this pathway during restraint stress does not prevent stress-induced anxiety. Ongoing studies using microdialysis combined with liquid chromatography-mass spectrometry examine how activation of this pathway changes downstream neurotransmission in the mPFC as a way to better understand how this projection drives these behaviors. Together these data suggest that stimulation of the LC-mPFC projection can promote avoidance to influence future behavior, but this projection may not initiate these behaviors in response to stress.

IMPLICATION OF MESOCORTICOLIMBIC DOPAMINE TRANSMISSION IN GOAL-DIRECTED BEHAVIORS: A ROLE FOR RECEPTOR HETEROMERS?

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Keywords : Dopamine ; Reward ; Heteromers ; Medial Prefrontal Cortex ; Nucleus Accumbens

Adaptive goal-directed actions is a key component of feeding behaviors. Mesocorticolimbic dopamine transmission is believed to be a key modulator of goal-directed actions and reward processing through its action on dopaminoceptive neurons expressing either D1 (D1R) or D2 receptors (D2R) in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc). Through chemogenetic approaches coupled with operant conditioning tasks, we demonstrated an implication of dopaminergic projections from the Ventral Tegmental Area in the flexible adaptation to a change in the instrumental action-outcome association. We also identified a complex interplay between D1R- and D2R-expressing neurons of the mPFC and the NAc in the flexible expression of food-oriented action as well as in the motivation to obtain a palatable reward. In both structures, the activity of dopaminoceptive neurons is strongly regulated by the convergence of glutamatergic and dopaminergic inputs. Heteromers formed by dopaminergic and glutamatergic N-methyl-d-aspartate receptors (NMDAR) recently emerged as molecular coincidence detectors of these transmissions, notably in the context of psychostimulant-induced adaptations. We therefore asked whether these receptor complexes could play a role in physiological reward processing. Expression of heteromers was mapped in substructures of the striatum and mPFC with Proximity Ligation Assay, allowing for the detection of specific antigen proximity on brain slices. Blockade of D1R-NMDAR or D2R-NMDAR heteromerization in either the mPFC or the NAc through the local viral-mediated expression of interfering peptides spared the main components of goal-directed behaviors, but selectively impaired the animals' ability to flexibly adapt to a change of contingency between action and outcome. These findings contribute to decipher the role of dopaminergic and dopaminoceptive neurons in executive functions and support a key role of heteromers formed by dopamine and NMDA receptors in flexible expression of food-oriented action.

COMBINING TEM AND NANOSIMS IMAGING TO DISCERN VESICLE COMPARTMENTS IN PC12 CELLS AND QUANTIFY VESICULAR ISOTOPIC DOPAMINE CONCENTRATION

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Keywords: NanoSIMS, TEM, dense core vesicle, dopamine

The absolute concentration and the compartmentalization of analytes in cells and organelles are crucial parameters in the development of drugs and drug delivery systems, as well as in the fundamental understanding of many cellular processes. Nanoscale secondary ion mass spectrometry (NanoSIMS) imaging is a powerful technique which allows subcellular localization of chemical species with high spatial and mass resolution, and high sensitivity.For this project, NanoSIMS imaging and transmission electron microscopy (TEM) imaging were combined to discern the compartments (dense core and halo) of large dense core vesicles (LDCVs) in PC12 cells and to localize ^{13}C dopamine enrichment following ^{13}C L-DOPA incubation. LDCVs are easily recognized in TEM images owing to their characteristic dense core structure. However, from a $^{12}C^{14}N$ NanoSIMS image alone, is it not possible to distinguish them from other cellular organelles and cytoplasmic features where the amount of $^{12}C^{14}N$ naturally differs. Here, by overlaying TEM images and the highly resolved $^{12}C^{14}N$ secondary ion images, different vesicle compartments could be localized and appeared as distinct based on their $^{12}C^{14}N$ content. In addition, the absolute concentrations of ^{13}C dopamine in these vesicle domains as well as in entire single vesicles were quantified and validated by comparing with electrochemical data. This confirms that NanoSIMS imaging can be used to carry out absolute quantification within highly resolved, subcellular biological compartments. This approach adds to the potential of using combined TEM and NanoSIMS imaging to perform absolute quantification and directly measure the individual contents of nanometer-scale organelles.

UNDERSTANDING EXTRASYNAPTIC TRANSMISSION IN THE BRAIN BY THE NEAR-INFRARED LIGHT TRIGGERED RELEASE

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Keywords: Plasmonic nanovesicles, near-infrared light, extracellular space, biotransport, brain

Understanding the role of neurochemicals on brain circuits and behaviour can be facilitated by the precisely controlled release of neurochemicals in the brain. Here, we synthesized a gold-coated mechanoresponsive nanovesicle, which consist of liposomes made from the artificial phospholipid Rad-PC-Rad as a tool for the delivery of bioactive molecules into the brain. Near-infrared picosecond laser pulses activated the gold-coating on the surface of nanovesicles, creating nanomechanical stress and leading to near-complete vesicle cargo release in sub-second. This high photosensitivity enables photorelease of molecules down to a depth of 4 mm in mouse brain. To investigate the neuropeptide volume transmission, we engineered plasmonic nanovesicles (Au-nV-SST) that can release somatostatin-14 (SST) with near-infrared light stimulation. To detect the functional response of SST, we created a nM sensitive cell-based neurotransmitter fluorescent engineered reporter (CNiFER) using the SST2 GPCRs. Using the combination of Plasmonic nAnovesicles and CNiFERs (PACE), we designed an integrated system to mimic neuropeptide volume transmission, including the release, diffusion, binding and signalling. Under intravital two-photon imaging, we measured the time for the released SST to activate SST2 GPCRs and subsequent signalling at defined distances. This measurement reveals synchronous transmission within 130 μm while a delayed transmission at larger distances. Our study provides the first quantitative estimation of the overall SST loss rate due to the degradation and binding in vivo, and demonstrates that neuropeptide binding and degradation limits its extrasynaptic transmission at large distances (>100 μm). We further demonstrated that SST transmission is significantly faster in neocortex with a chemically degraded extracellular matrix. The PACE technique reveals the spatiotemporal scales of neuropeptide volume transmission and signalling in the brain and provides a useful tool to investigate important physiological processes in living systems.

DOPAMINE RELEASE DRIVEN BY CLASSICAL CONDITIONING OF VISUAL STIMULI IN THE SUPERIOR COLLICULUS

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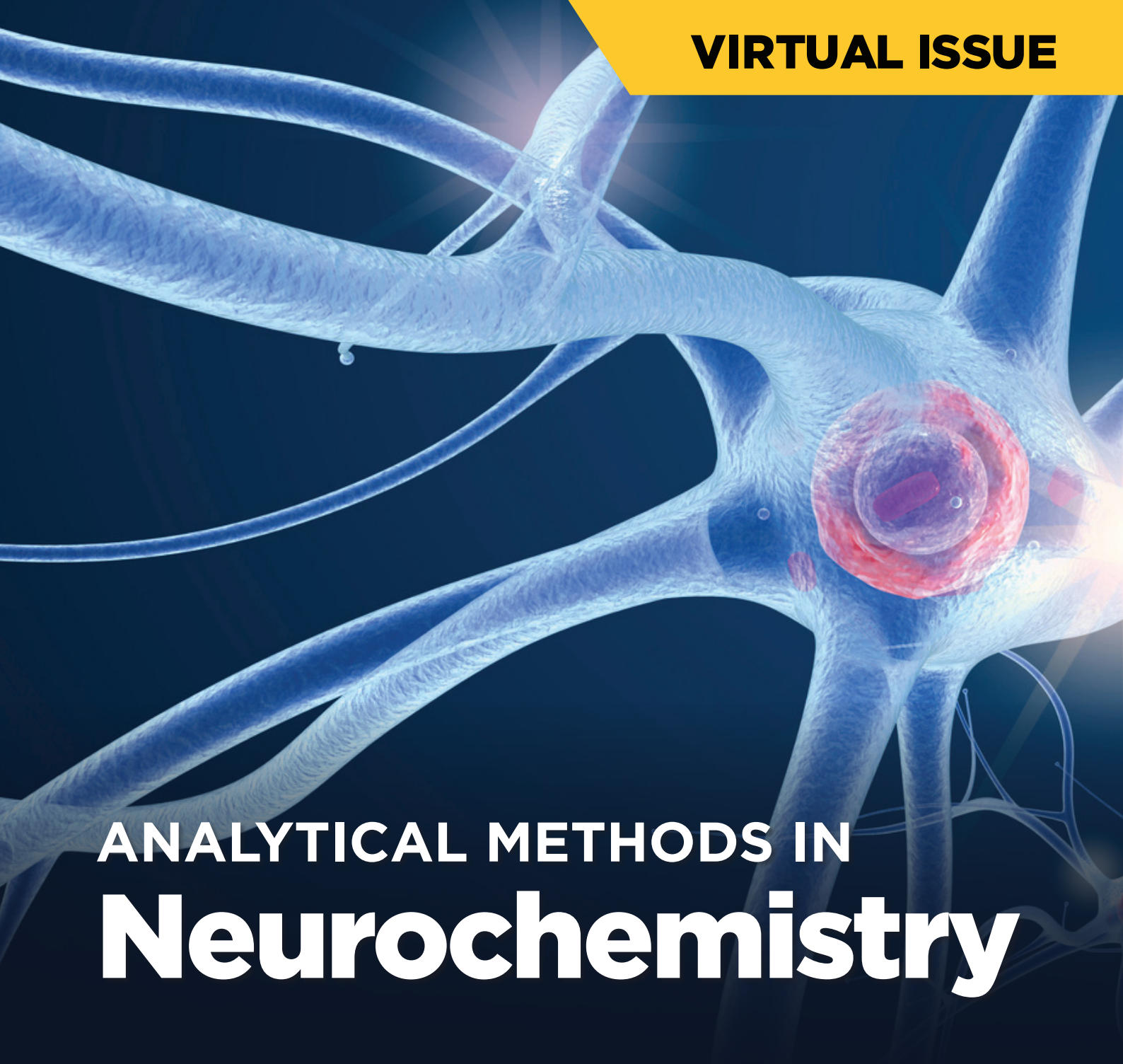
Keywords: Visual classical conditioning, superior colliculus, phasic dopamine activity, serotonin, learning

Activation of dopamine neurons in the midbrain by stimuli conditioned to predict reward is a well-established element of classical Pavlovian conditioning. However, how the dopamine neurons come to report the association between a conditioned stimulus (CS) and a reward remains unknown. Here we show that the superior colliculus (SC) develops neuronal responses to a visual CS during conditioning, and in turn drives the short-latency responses of dopamine neurons. Visual responses in the SC were only potentiated when a behaviourally meaningful time interval separated the visual stimulus and reward. Potentiation also required the convergence of visual, dopamine and serotonin inputs to the SC. Importantly, blocking potentiation of the visual response was sufficient to suppress the release of dopamine in the striatum following a CS. These results reveal a mechanism for how the brain forms associations between unconditioned stimuli and behaviourally meaningful visual information during classical conditioning.



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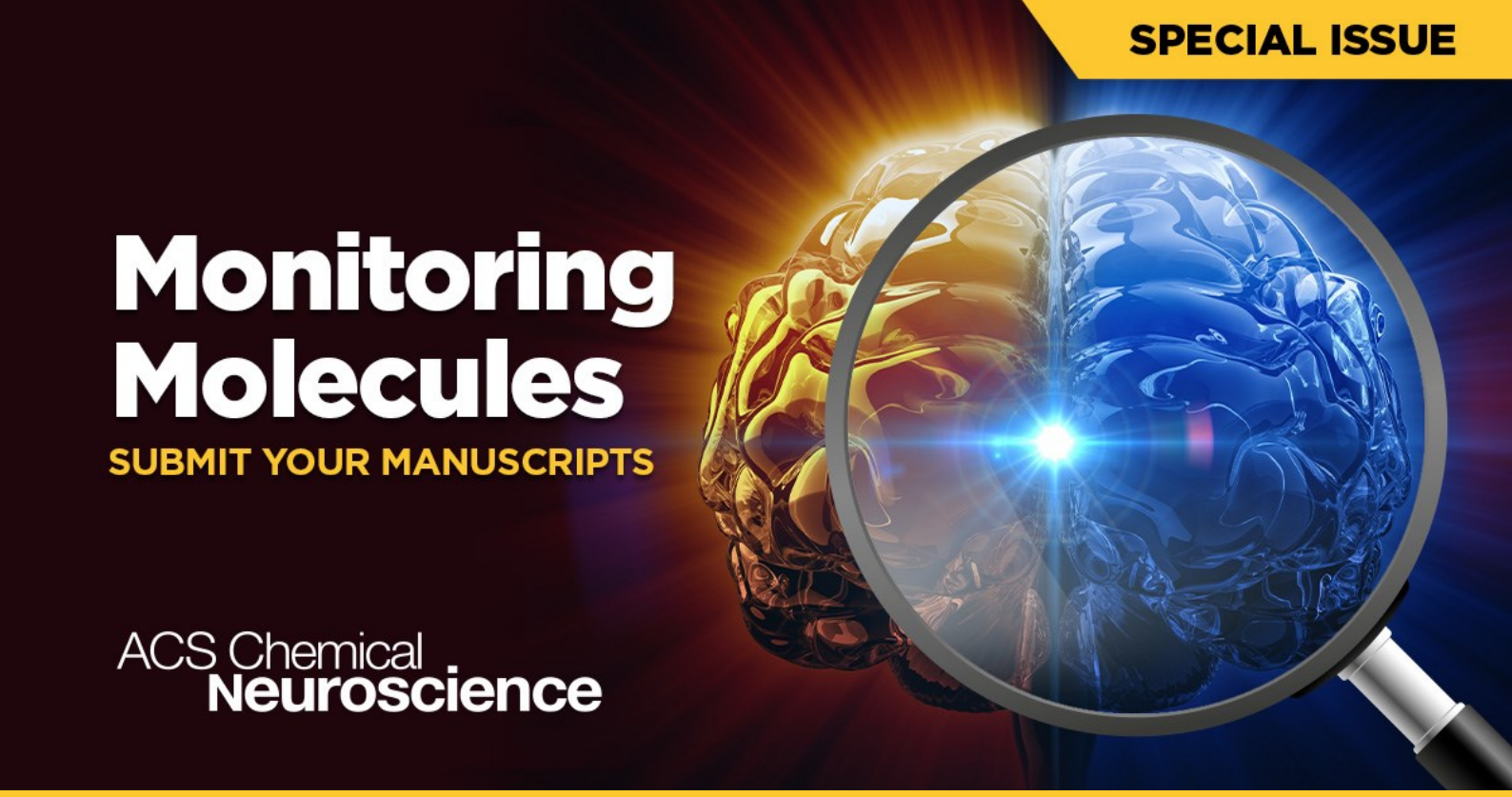

Prof. Leslie A. Sombers, ACS Chemical Neuroscience Associate Editor and MMiN Advisory Board Member,

is pleased to share with you that **ACS Chemical Neuroscience** will publish its **5th special issue on Monitoring Molecules in Neuroscience**.

All meeting participants are invited to submit Articles and Letters, as well as Reviews, Perspectives, and Viewpoints that chart the future of the field.

Ideas for submissions can be discussed in advance (sombers-office@chemneuro.acs.org)

Deadline to submit is **November 1, 2022**.

Accepted papers are published online ASAP; the curated special issue will appear in the **first half of 2023**.

MMiN 2024

Chapel Hill, NC, USA

May 2024 (Exact dates TBC)

Sara R. Jones
Co-chair

Zoe A. McElligott
Co-chair

MMiN
International Society for
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