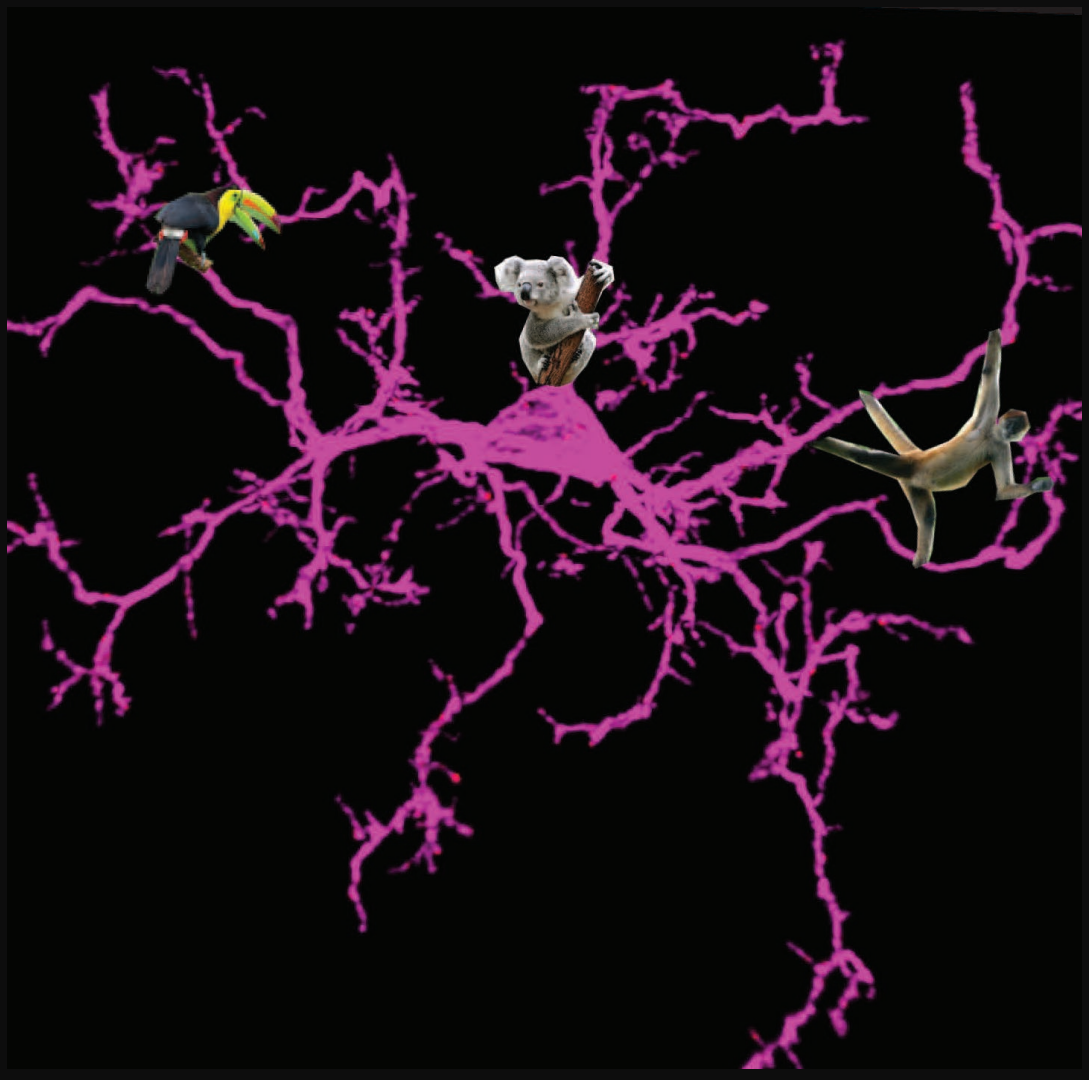
The Program in Neuroscience Presents:



The 24th Annual Retreat

Illustration

Cover microglial image by *Benjamin Siemsen, Research Associate, Lobo lab*

Special Thanks

Pre-doctoral Neuroscience Training Program
(PI: Jessica Mong)

And, the continued support of the School of Medicine, the Dental School, and the Graduate School.

About the Program in Neuroscience

The Graduate Program in Neuroscience (PIN) is an interdisciplinary program of study leading to a PhD degree in Neuroscience. For over 20 years, PIN has been a center of excellence for graduate training in the life sciences. PIN faculty expertise and research interests range from molecular to clinical realms. Our Program enhances interaction among our internationally renowned faculty and enables graduate students to take advantage of the full depth and breadth of Neuroscience research conducted at the University of Maryland, Baltimore. Our PhD students are highly sought after, routinely being appointed as postdoctoral fellows at other prestigious academic institutions or finding employment at one of the many Neuroscience-related occupations in industry and government, including the many options in the greater Washington D.C./Baltimore area.

To learn more about our program please visit:
https://lifesciences.umaryland.edu/neuroscience/

PIN Gratefully Acknowledges

- **Brian Mathur** for his outstanding service as Director of Graduate Education and for chairing the PIN Training Committee
- **Margaret McCarthy** for her phenomenal service as the Director of the Program in Neuroscience
- **Renee Cockerham** for coordinating PIN while attending to postdoctoral affairs
- **Jennifer McFarland** for coordinating PIN
- **Marta Lipinski** for coordinating the Neuroscience Journal Club
- **Norbert Myslinski** for his work on the International and National BrainBee
- **Donna Calu** for chairing the Retreat Committee
- **Marco Venniro** for chairing the Seminar Committee
- **Mary Kay Lobo** for chairing the Diversity Committee
- **Ashley Marquardt** for chairing the PIN Student Training Committee, serving as the student representative on the PIN training committee, and managing the @UMMedNeuro PIN Twitter feed
- **Emily DeMarco and Katia Matychak** for serving on the Graduate Student Association (GSA)
- **Sydney Ashton** for serving as the President of GSA and as the student representative on the Seminar Committee
- **Cali Calarco** for serving as the Postdoctoral Representative on the Seminar Committee
- **Garrett Bunce** for serving as the President of NOVA (Neuroscience Outreach and Volunteer Association)
- **Sarah Keefer, Rachel Cundiff-O'Sullivan, and Andreas Wolff** for organizing the UMB Baltimore Brain Series
- **Dudley Strickland and Sharron Graves** for leadership and service to the Graduate Program in Life Sciences (GPILS)
- Faculty, Students and Staff whose time and effort ensured the recruitment of an outstanding group of new PIN students
- Faculty course directors and instructors
- Student and postdoc volunteer educators
- **And most of all, PIN graduate students and postdocs**

PIN Standing Committees

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Jennifer McFarland
Soad Elziny
Cody Scholtens
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Melanie Pina

The 24th Annual Program in Neuroscience Retreat

Tuesday, June 7th, 2022
Mansion House at the Maryland Zoo
1876 Mansion House Dr., Baltimore, MD 21217

9:00-9:30 **Registration** (Coffee & light snacks provided)

9:30-9:40 **Introductory Remarks** (Donna Calu & Brian Mathur)

9:40-10:00 **Research Talks** (8 min talks/2 min questions):

Abigail Vigderman, Graduate Student, Longden Lab
"Measurement of Cerebrovascular Hemodynamics in Awake Freely Behaving Mice"

Lan-Yuan Zhang, Postdoctoral Fellow, Cheer Lab
"Astrocytic CB1 receptor signaling in the ventral tegmental area is required for food-driven motivation"

10:00-10:05 **Faculty video and 1st year student video**

10:05-10:20 **PIN director Peg McCarthy Remarks and Awards**
PIN Awards and J. Tyson Tildon Award

10:20-10:25 **Coffee Break** and Breakout Session Transition

10:25- 11:00 **Poster Jam Session**:
Breakout Session: 3-min-thesis style presentations

11:00-12:00 **Keynote Address: Mollie Meffert, "Growth regulatory microRNAs in neurodevelopment and plasticity"**

Dr. Meffert is an Associate Professor, Departments of Biological Chemistry and Neuroscience; Vice Director, Department of Biological Chemistry at The Johns Hopkins University School of Medicine

12:00-1:00 **Lunch indoors or outdoors at Pavilion**

1:00-2:00 **Pavilion Social hour**

2:00- 3:15 **Activities, Scavenger hunt and/or lawn Olympics, and Awards**

3:15-4:00 **Leisure and Retreat Closing**

J. Tyson Tildon Award for a Promising Neuroscientist and Community Servant

In memory of J. Tyson Tildon and his commitment both to neuroscience research and service to his community, the J. Tyson Tildon Award for a Promising Neuroscientist and Community Servant will be given, periodically, to a dynamic postdoctoral fellow who has demonstrated community service. This is a cash award to help the recipient launch her/his career.

Dr. Tildon was the founder and head of the Division of Pediatric Research and first Associate Dean for Research for the University of Maryland School of Medicine. Dr. Tildon also served on boards for the Baltimore City Public Schools, United Way of Maryland, Associated Black Charities, WYPR radio, the Civil Services Commission of Baltimore, the Maryland Academy of Sciences, the Enoch Pratt Free Library and the American Red Cross.

Nominee Eligibility Criteria

The postdoctoral fellow chosen to receive this award will have demonstrated a history of critical thinking and a passion for continued learning in neuroscience. Additionally, the fellow will have a proven commitment to helping others in the scientific community or community at large, reflecting the spirit of Dr. Tildon's dedication to the pursuit of research and exceptional service.

Nomination process

To be nominated for this award, the mentor must have known the postdoctoral fellow for at least one year in order to gauge her/his character, ability, and commitment to service. The mentor must submit a nomination letter, the nominee's CV, a brief personal statement written by the nominee (no more than one page), and 1-2 other supporting letters. At least one supporting letter must be from someone who can directly address the fellow's service contributions.

Nomination deadline

All materials should be submitted to the Director of the Neuroscience Program by April 15.

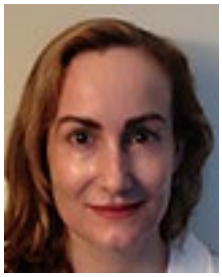
Award announcement

The award will be presented at the Annual Program in Neuroscience Retreat the following June.

Award conditions

This award is to help the recipient launch her/his career. For example, it can be used by the recipient to travel to a conference to present her/his research and gain valuable networking, or to train in specific methodology by working in the expert in the field After one year, the awardee will be expected to submit a brief statement of how the award was used to benefit her/his career.

Keynote Speaker



Mollie K. Meffert, M.D., Ph.D., M.S., *Associate Professor, Departments of Biological Chemistry and Neuroscience; Vice Director, Department of Biological Chemistry at The Johns Hopkins University School of Medicine*

Dr. Mollie Meffert is an associate professor of biological chemistry and neuroscience at the Johns Hopkins University School of Medicine. Her research focuses on the regulation of neuronal gene expression in health and disease.

Dr. Meffert received her undergraduate degree from Stanford University. She also earned her MD/Ph.D in neuroscience from Stanford University. She completed a postdoctoral fellowship at California Institute of Technology with Dr. David Baltimore. Dr. Meffert currently serves as the Vice-Director of the Department of Biological Chemistry

The Meffert lab studies gene control mechanisms underlying enduring changes in brain function. We are interested in understanding how programs of gene expression are coordinated and maintained to produce altered synaptic, neuronal, and cognitive function. Rather than concentrating on single genes, our research is particularly focused on revealing upstream processes that can perform synchronous up and down-regulation of the many genes required to orchestrate physiological responses.

Our laboratory elucidated a post-transcriptional mechanism capable of organizing pro-growth gene programs in which activity-dependent regulation of microRNA (miRNA) production governs the selection of gene targets for protein synthesis. An RNA-binding protein, Lin28, is one activity-responsive factor that promotes pro-growth protein synthesis by downregulating only select miRNAs (e.g. Let-7 'tumor suppressor' miRNAs), which repress pro-growth genes. In neurons, pro-growth mRNA targets of the Let-7 miRNAs include mRNA for proteins involved in excitatory synaptic function, as well as growth and repair. An ongoing focus of investigations in our laboratory is aimed at further exploration of the importance of miRNA biogenesis in determining rapid and specific changes in the neuronal and synaptic proteome and the in vivo roles of these pathways in healthy and dysregulated brain function.

Dr. Meffert's work has been recognized with a number of awards including the March of Dimes research scholar, a Simons Foundation Autism Research Initiative award, the PLU Rho Award, the Alfred P. Sloan Research Fellow Award, and the Sontag Foundation Distinguished Scientist Award.

Research Talks

Measurement of Cerebrovascular Hemodynamics in Awake Freely Behaving Mice

Abigail S. Vigderman, Graduate Student, Longden Lab

The mechanisms by which neurons communicate activity-dependent energy needs to the surrounding vasculature to evoke an increase in blood flow (functional hyperemia) are collectively termed neurovascular coupling (NVC). This hyperemic response is frequently used as a readout for neuronal activity in functional imaging studies, but these are typically limited by the need for immobilization of the subject inside a scanner. A range of techniques have been utilized to measure these hemodynamic changes resulting from NVC, but to date none have provided precise, cellular-resolution data in awake and freely behaving animals, which is necessary to gain a full understanding of brain blood flow control in health and disease. We combined novel in vivo imaging technologies to measure brain blood flow at the single capillary level using miniature head-mounted microscopes (miniscopes) and compare this to the current gold-standard multiphoton imaging methods for measurement of cerebral blood flow. We combine the use of miniscopes for data collection with automated analysis for rapid, accurate, and impartial assessment of blood flow through large and complex vascular networks in deep brain structures with high spatiotemporal precision. By adapting miniscopes to study hemodynamics in detail, we aim to ultimately map blood flow through local networks during NVC and functional hyperemia to provide a window into the dynamics of these mechanisms in awake, freely behaving animals. As blood flow disruption is one of the earliest events in dementia and a strong predictor of cognitive decline, refinement of these approaches may eventually aid in developing diagnostic tools aiming to detect subtle early changes in blood flow that, if left unchecked, may precipitate neuronal dysfunction.

Astrocytic CB1 receptor signaling in the ventral tegmental area is required for food-driven motivation

Lan-Yuan Zhang, Postdoctoral Fellow, Cheer Lab

Cannabinoid type I receptors (CB1R) modulate dopamine (DA) neuron activity in the ventral tegmental area (VTA), a midbrain structure of the mesolimbic system critical for motivation and rich in astrocytes. In the hippocampus and nucleus accumbens (NAc), astrocytic CB1R activation modulates neuronal plasticity by promoting calcium (Ca2+) transients and release of gliotransmitters. Whether VTA astrocytes express CB1R to modulate accumbal dopaminergic correlates of motivated behavior is unknown. Here, we present evidence that CB1R are indeed abundantly expressed on VTA astrocytes. Using fiber photometry in freely behaving mice, we show that conditional, inducible CB1R deletion in VTA astrocytes elevated astrocytic Ca2+ transients, reduced motivation for food reward and inhibited reward-evoked DA release in the NAc. These findings establish CB1R on astrocytes in the VTA as critical regulators of mesolimbic dopaminergic projections recruited during the motivated pursuit of reward.

Posters and Short Presentations

Olfaction drives socio-sensory buffering of drug choice

Kimberly M. Papastrat[1] , Adam C. Puche[1] , Marco Venniro[1]

[1]Department of Anatomy & Neurobiology, University of Maryland School of Medicine, Baltimore, MD, USA

Background: Volitional social interactions with peers are highly rewarding and can be used as a buffer against abused drugs. Organisms across all species require sensory systems to capture emotions, communicate during social interactions, and share information about the surrounding environment.

Methods: First, we tested the role of olfactory system in either acquisition or maintenance of volitional social interaction. Using our volitional social-choice self-administration rat model, we trained male and female rats for either food (2-h/d, 5-d) or social self-administration (2-h/d, 10/12-d). To test the role of olfaction, we removed the olfactory bulbs (bulbectomy – or sham surgery) before (experiment 1) or after (experiment 2) acquisition of operant social behavior. Next, we tested the role of the olfactory system in social choice-induced inhibition of drug self-administration. After training bulbectomy or sham rats for social (2-h/d, 10-d) and cocaine (6-h/d, 12-d) self-administration, we introduce a choice between social interaction and cocaine.

Results: In both sexes bulbectomy selectively prevented acquisition and maintenance of social interaction, although the other sensory components were intact. Bulbectomized rats exhibited reliable food and cocaine self-administration, showing motivation for other rewards. Rats with an intact olfactory system showed strong social preference over cocaine. However, cocaine self-administration resumed in rats deprived of the olfactory system.

Conclusion: We identified the olfactory system as the primary driver of socio-sensory communication mediating volitional social interaction and of the protective effect of social reward on drug choice. From a translational perspective, these findings highlight the critical importance of sensory cues during peers' communication for implementation of social-based addiction treatments.

Abnormal Brain Diffusivity in Participants with Persistent Neuropsychiatric Symptoms after COVID-19

Huajun Liang MBBS, PhD[1], Thomas Ernst PhD[1,2], Kenichi Oishi MD, PhD[3], Meghann C. Ryan MA[4], Eric Cunningham BS[1], Eleanor Wilson MD[5], Andrea Levine MD[6], Shyamasundaran Kottilil MD, PhD[5], Linda Chang MD[1,2,7]

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[5]Institute of Human Virology, Department of Medicine, Division of Infectious Disease, University of Maryland School of Medicine, Baltimore, MD, United States.
[6]Department of Medicine, Division of Pulmonary & Critical Care Medicine, University of Maryland School of Medicine, Baltimore, MD, United States.
[7]Department of Neurology, University of Maryland School of Medicine, MD, United States.

Background: Neuropsychiatric symptoms of post-acute sequelae of COVID-19 (PASC) are common during convalescence. Abnormal brain white matter integrity was found within 6 months after acute infection in those who recovered from COVID-19, but these studies did not specifically evaluate those with neuropsychiatric symptoms.

Methods: Cognitive performance, psychiatric symptoms (NIH Toolbox® and PROMIS), and diffusion tensor imaging (DTI) metrics were compared for 23 participants with PASC (15 women, average age=44.1±2.5 years, 6 months since COVID-19) and 24 uninfected controls (13 women, average age=44.3±2.6 years). Fractional anisotropy (FA), axial (AD), radial (RD), and mean (MD) diffusivities were assessed using MRICloud with an automated Multi-atlas label fusion method to evaluate 9 white matter and 6 subcortical brain regions.

Results: Compared to controls, PASC had similarly normal cognitive performance, but greater psychiatric symptoms and perceived stress, higher FA, and lower diffusivities in multiple white matter tracts on the right and few on the left (ANCOVA-p-values=0.004-0.048). Women with PASC had higher left amygdala-MD compared to control women ($p=0.010$), but no difference in men (Sex-by-PASC-$p=0.006$). Higher sagittal strata-FA predicted greater fatigue in all participants ($r=0.515$, $p<0.001$). Regardless of COVID-19 status, higher left amygdala-MD predicted greater fatigue ($r=0.613$, $p<0.001$) and anxiety ($r=0.687$, $p<0.001$) in women and higher perceived stress ($r=0.449$, $p=0.002$) across all participants.

Conclusions: PASC had more microstructural abnormalities including restricted white matter diffusivity and higher amygdala diffusivity, a consistent finding among survivors of traumatic stressors. This suggests COVID-19 might have exacerbated the stress reaction in survivors and contributed to the persistent neuropsychiatric symptoms.

Overexpression of the proSAAS chaperone reduces levels of endogenous □-synuclein and sequesters preformed synuclein fibrils.

Kriti Chaplot and Iris Lindberg

University of Maryland Baltimore

Chaperone proteins perform critical functions against aberrant protein aggregation involved in neurodegeneration. The abundantly-expressed neuronal chaperone proSAAS has been found to be associated with Lewy bodies, beta amyloid plaques, and tau tangles in patient samples. In collaboration with Maidment lab, we have found that virally-expressed nigral proSAAS provides profound protection from motor asymmetry in a rat model of Parkinson's disease (PD). Here, we have investigated various mechanisms by which proSAAS might exert its cytoprotective effects.

Upon testing whether proSAAS can impact α-synuclein (α-syn) metabolism in cells, we found that viral proSAAS overexpression reduces endogenous α-syn protein levels in primary rat hippocampal cells in a dose-dependent manner, suggesting a potential mechanism for improvement of motor deficits in the rat PD model due to the loss of contributing endogenous α-syn substrate. Studies are now in progress to determine the effect of proSAAS on possible degradative mechanisms involved in α-syn reduction.

Past *in vitro* studies have shown that proSAAS acts as a chaperone for α-syn by reducing the fibrillation of monomeric α-syn at low stoichiometric ratios, but whether proSAAS can also block the formation of higher order oligomers is unclear. We now report that recombinant proSAAS does not reduce α-syn fibrillation in reactions seeded with sonicated pre-formed α-syn fibrils (PFFs), suggesting that proSAAS chaperone action likely occurs only at the monomer to oligomer transition rather during secondary nucleation and fibril elongation.

We have also previously shown that proSAAS, a secreted molecule, blocks the transmission of human synuclein across synapses. To examine proSAAS effects on acute cellular uptake of PFFs, we prepared fluorescent PPFs from α-syn tagged with GFP via a linker sequence containing the Tobacco Etch Virus (TEV) protease cleavage site ("GFP-PFFs"). Following TEV treatment to remove external GFP-PFFs, we specifically assessed cell-associated and/or internalized GFP-PFFs in the presence or absence of added recombinant proSAAS. Confocal imaging revealed that complexes of proSAAS and GFP-PFFs precipitate on the cell membrane (as opposed to the GFP-PFFs combined with the inert control protein, ovalbumin, which are internalized). In agreement, the percent of GFP-positive cells (as estimated by flow cytometry) and the quantity of GFP-PFFs within cell lysates (by Western blotting) are higher in the presence of proSAAS vs ovalbumin, supporting the idea that proSAAS acts extracellularly to sequester aggregating species.

Taken together, our results suggest that the proSAAS chaperone performs cytoprotective functions via three distinct mechanisms- it reduces cellular α-syn protein levels; it blocks monomeric α-syn fibrillation; and it acts as a sequestrase for oligomeric and aggregating forms of α-syn.

An optical analysis framework for measuring physiology at single synapses using GluSnFR3

Samuel T. Barlow and Thomas A. Blanpied

Department of Physiology, School of Medicine, University of Maryland Baltimore

Over the last decade, rapid progress in the development of fluorescent protein sensors for neurotransmitter release have made optical measurements of synaptic physiology, and in particular, presynaptic activity, possible at the level of individual synapses. Here we use the newest version of the intensity-based glutamate sensing fluorescent reporter (GluSnFR3) to measure glutamate release at individual synapses in cultured rat hippocampal neurons, aiming to extract functional properties (e.g. release probability, frequency, and quantal content) of glutamate release during electrical stimulation or action potential-independent paradigms.

GluSnFR3 enables the measurement of release properties at hundreds of synapses in parallel, but extracting information from these optical recordings in an efficient and unbiased manner requires the development of new analysis frameworks. Here we present an analysis pipeline which extracts intensity-time traces from automatically-segmented, putative synapses according to their GluSnFR3 activity. As regions-of-interest display broad variation in noise levels and baseline stability, we implement an iterative outlier detection approach which enables flexible identification of the baseline across a variety of trace conditions and improves the accuracy and precision with which we can determine GluSnFR3 $\Delta F/F$. After peak identification, we implement an exponential decay fitting routine for peak quantification and post-hoc quality control. By deploying these routines in parallel with spatial analysis, we can characterize glutamate release characteristics at identified synapses in cultured neurons, including identification of asynchronous release events, subsynaptic release site position, and correlation with concurrent optical readout of postsynaptic activity.

Impact of ionotropic glutamate receptors on trans-synaptic nanostructure

Poorna A. Dharmasri, Michael C. Anderson, Aaron D. Levy, and Thomas A. Blanpied

University of Maryland Baltimore School of Medicine, Dept of Physiology

Our lab has demonstrated a subsynaptic coordination between pre- and postsynaptic protein organization, termed the *trans-synaptic nanocolumn*. This nanocolumn is critical for synaptic transmission - dispersal of AMPARs from the nanocolumn to the remainder of the synapse specifically reduces the strength of the response to evoked release. This fundamental role of the nanocolumn motivates further understanding of how trans-synaptic alignment is achieved. We reasoned that a mechanism underlying trans-synaptic alignment would involve a protein that acts either as a structural or functional marker of successful transmission. Intriguingly, the iGluRs embody both aspects. Either iGluR could trigger downstream mechanisms that sample receptor activation to drive alignment. Both AMPARs and NMDARs have extended extracellular structures with distal N-terminal domains that could nucleate direct or indirect interaction with presynaptic proteins. iGluRs could also organize scaffold proteins through multivalent intracellular interactions and previous studies show that receptor structure influences synaptogenesis and presynaptic maturation. Thus, we hypothesized that the iGluRs align the trans-synaptic nanocolumn. We tested nanoscale organization of presynaptic vesicle docking protein Munc13 and postsynaptic scaffold PSD95 in individual knockouts of the GluA2 AMPAR subunit and NMDARs in rat primary hippocampal culture. We report that loss of iGluRs, even just GluA2, results in changes to pre- and postsynaptic nanoscale organization and strongly disrupted alignment. On-going experiments will elucidate the structural or functional influence of iGluRs. This study challenges the thought that iGluRs are organized downstream of pre-existing scaffold structure, presenting evidence that they actively shape synaptic structure and thus may dictate their own activation.

Central amygdala transcriptional profile mediating volitional social interaction and psilocybin treatment

Alexandra Fall and Marco Venniro

Program in Neuroscience, Department of Anatomy and Neurobiology, University of Maryland School of Medicine

Social support has strong protective effects on drug taking and seeking for both humans and laboratory animals. Recently, it has been reported that psychedelics have strong protective effects against different neuropsychiatric disorders, including substance use disorder. However, it is unknown whether the protective effect of social interaction and psychedelics on drug craving share common mechanisms. Here, we start our investigation to address this gap. We have recently showed that the central amygdala is critical for the protective effect of social interaction on drug craving. Additionally, psychedelics have been shown to induce epigenetic remodeling and synaptic plasticity. Here, we investigated transcriptional changes within the central amygdala induced upon either volitional social interaction or psilocybin treatment. First, we trained male and female rats first for food (60s, 2h/d, 4d) and then for social (60s, 2h/d, 10d) self-administration. In a different group of male and female rats, we administered (IP) psilocybin at either 1 mg/kg or 5 mg/kg. We isolated RNA from the central amygdala at 5, 9, and 15 days after social self-administration and 24 h or 9 days after psilocybin treatment. Bulk RNA sequencing will be used to probe alterations in the central amygdala transcriptome after social self-administration or psilocybin treatment.

Cholesterol accumulation in lysosomes impairs autophagy in mononuclear phagocytes after traumatic brain injury and contributes to neuroinflammation

Amir A Mehrabani-Tabari[1], Nivedita Hegdekar[1], Yulemni Morel[2], Ludovic Muller[2], Chinmoy Sarkar[1], Mureen A. Kane[2], Jace W. Jones[2], and Marta M. Lipinski[1]

1. University of Maryland Baltimore School of Medicine Department of Anesthesiology, Shock, Trauma, and Anesthesiology Research (STAR), 2. University of Maryland Baltimore School of Pharmacy Department of Pharmaceutical Sciences

Prolonged neuroinflammation, mediated by both resident microglia and infiltrating monocytes/macrophages, has been consistently observed in traumatic brain injury (TBI) and correlated to poor prognosis. Data from our laboratory demonstrate that inhibition of the catabolic pathway autophagy, in both activated microglia and infiltrating macrophages contributes to their proinflammatory phenotype in TBI lesion. However, the mechanisms responsible for inhibition of autophagy in monocytes after TBI remain unknown. Our data, including LC-SM/MS lipidomic analysis and immunofluorescent staining demonstrate that inhibition of autophagy in infiltrating macrophages after TBI is associated with intracellular lipid accumulation and formation of lipid droplets. The phenotype resembles foam macrophages also observed in multiple sclerosis where it is thought to be caused by phagocytosis of myelin-derived cholesterol, which is abundant in TBI lesion. Cholesterol accumulation has been shown to disrupt lysosomal function in various tissues. We hypothesized that excessive uptake of myelin debris and other complex cholesterol species such as oxidized LDL (oxLDL), which we also observed to be abundant in TBI brain, leads to inhibition of autophagy after TBI. Consistently, we observed intracellular lipid accumulation and pronounced inhibition of autophagy in RAW macrophages exposed to several different cholesterol species including oxLDL. Cholesterol exposure also exacerbated LPS-induced inflammatory responses. Our data also confirmed colocalization of lipid accumulation with lysosomal marker in monocytes in TBI brain sections. Our data support a mechanism where lipid and cholesterol accumulation in myeloid cells leads to lysosomal dysfunction and inhibition of autophagy, thus exacerbating TBI inflammatory responses.

Multiplexed manipulations of gene dosage of schizophrenia risk genes using Cas9 fusions changes layer position of cortical neurons.

Andrea Romanowski, Bekir Altas, Ryan Richardson, Saovleak Khim, Alexandros Poulopoulos

Department of Pharmacology, University of Maryland School of Medicine, Baltimore, MD

Neuropsychiatric disorders are highly heritable, with estimates ranging from 50-80% in schizophrenia and autism. Large scale genome sequencing efforts suggest that the majority of non-syndromic cases have polygenic underpinnings with variants in regulatory non-coding regions of risk genes. This suggests that common variants associated with schizophrenia risk manifest through changes of gene regulation, affecting timing and dosage of risk genes rather than affecting protein structure or function. In order to model polygenic schizophrenia risk gene variation in the rodent brain, we have developed an in utero electroporation approach using Cas9 fusions to investigate what effects changing expression levels of multiple risk genes has on circuit development. Using multiple gRNAs, we changed gene dosage of genes in the immunoglobulin cell adhesion superfamily implicated in risk for schizophrenia and other neuropsychiatric conditions. We demonstrate that dosing multiple genes in the same neuron affects it's layer positioning in the sensorimotor cortex. To investigate the molecular mechanisms leading to these effects during early postnatal brain development, we epitope tag the endogenous gene products using Cas9-RC, a high-performance in vivo knockin CRISPR agent. Using this approach we will compare the subcellular localization and interactions of endogenous versus dose-manipulated gene products during cortical neuron migration. The broader aim of these approaches is to develop a workflow for manipulating gene dosing in multiple risk genes in the developing brain to investigate impacts on cortical wiring across neurodevelopmental and neuropsychiatric conditions.

Differential Claustrum Responses to Experimental Pain in Chronic Pain Patients and Healthy Controls

Brent W. Stewart[1], Suk W. Han[2], Michael Keaser[1], Hwiyoung Lee[3], Shuo Chen[3], Brian Mathur[4], David Seminowicz[1]

1. Dept. of Neural and Pain Sciences, University of Maryland Baltimore
2. Dept. of Psychology, Chugnam National University
3. Dept. of Epidemiology & Public Health, Maryland Psychiatric Research Center
4. Dept. of Pharmacology, University of Maryland Baltimore

The claustrum is a telencephalic structure with widespread cortical connectivity and undetermined function. Numerous hypotheses exist for claustrum function, including salience detection and cortical network instantiation for cognitive control. In humans, the claustrum exhibits difficulty-dependent activation at the onset of a cognitive task, a stimulus known to induce synchronous activity of a task-positive cortical network. The following analysis aimed to test predictions of the network instantiation hypothesis by examining claustrum responses to another stimulus known to induce functional connectivity of a cognitive task-associated network, experimental pain. Given established differences in cortical network dynamics between healthy controls and chronic pain patients, samples of both populations were tested. Additionally, the salience detection hypothesis was tested in another dataset of healthy participants via examination of claustrum responses to task-irrelevant, affectively salient "oddball" videos. In the pain experiment, anterior cingulate cortex and insular cortex salience network nodes exhibited characteristic responses to painful stimulation. Healthy controls exhibited significantly increased left, but not right, claustrum activity at pain stimulus onset, whereas chronic pain patients exhibited increased bilateral claustrum activity at pain stimulus onset. In the oddball experiment, significant activation was detected in left anterior insula, but no significant signal change was observed in the dorsal anterior cingulate cortex, left claustrum, or right claustrum in response to the oddball stimulus. Collectively, these findings are consistent with the network instantiation model of claustrum function and inconsistent with the salience detection model, and they suggest the claustrum as a possible therapeutic target for chronic pain conditions.

The mouse claustrum synaptically connects cortical network motifs

Houman Qadir[1], Brent W. Stewart[2], Jonathan W. VanRyzin[1], Qiong Wu[4], Shuo Chen[3], David A. Seminowicz[2] and Brian N. Mathur[1]

[1]Department of Pharmacology, University of Maryland School of Medicine, Baltimore, MD, USA.
[2]Department of Neural and Pain Sciences, University of Maryland School of Dentistry, Baltimore, MD, USA.
[3]Division of Biostatistics and Bioinformatics, Department of Epidemiology & Public Health, University of Maryland School of Medicine, Baltimore, MD, USA.
[4]Department of Biostatistics, Epidemiology and Informatics, University of Pennsylvania, Philadelphia, PA, USA.

Spatially distant areas of cerebral cortex coordinate their activity into networks that are integral to cognitive processing. A common structural motif of cortical networks is co-activated frontal and posterior cortical regions. Knowledge of the neural circuit mechanisms underlying such widespread inter-areal cortical coordination is lacking. Using anesthetized mouse functional magnetic resonance imaging (fMRI) we discovered that mouse frontal cortical functional connectivity reflects the common cortical network motif in its functional connectivity to posterior cortices, but also demonstrates significant functional connectivity with the claustrum. Exploring whether the claustrum may synaptically support such network architecture, we used a channelrhodopsin-assisted electrophysiological circuit mapping approach to assess the strength of synaptic connectivity of 35 unique frontal cortico-claustral-cortical connections through 1,050 subtype-identified claustrum projection neurons. We observed significant trans-claustral synaptic connectivity from the anterior cingulate cortex and prelimbic prefrontal cortex back to originating frontal cortical regions as well as to posteriorly-lying visual and parietal association cortices contralaterally. The infralimbic prefrontal cortex possessed significant trans-claustral synaptic connectivity with the posteriorly-lying retrosplenial cortex, but to a far lesser degree with visual and parietal association cortices. These data reveal discrete extended cortical pathways through the claustrum that are positioned to support cortical network motifs central to cognitive control functions.

The role of parabrachial in nociception and pain in awake mice

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The parabrachial nuclear complex (PB) is a nexus for aversion, and for the nociceptive and affective components of pain perception. We have previously shown that, during chronic pain, PB neurons have increased activity and respond to noxious stimuli with prolonged after-discharges – responses that far outlast the stimulus. This phenomenon—like most of what we know about the electrophysiology of pain—has only been observed in anesthetized animals. Anesthesia profoundly alters neuronal responses to nociception and masks their responses to the affective component of pain. We have developed a method to investigate PB in awake, behaving animals by recording single units *in vivo* from head restrained mice. This offered opportunities to study the time course of changes in PB activity by recording repeatedly from the same animals. It also allows us to correlate PB activity with the animal's behavioral state, by using pupil changes as a proxy for internal states. We report that, in PB neurons from both male and female mice, anesthesia leads to decreased activity, specifically a decrease in spontaneous activity and reduced magnitude of the responses to noxious stimuli. We also demonstrate that, in awake mice, evoked response both before and after chronic pain results in a lasting amplification of PB activity. Finally, we show that changes in PB activity are related to changes in arousal, which was captured by increases in pupil diameter states.

This work was supported by National Institutes of Health National Institute of Neurological Disorders and Stroke Grants R01NS099245 and R01NS069568.

Serotonergic Modulation of the Claustrum

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Cognitive flexibility deficits are a major contributor to diminished life and therapeutic outcomes across myriad neuropsychiatric disorders including Alzheimer's, depression, and schizophrenia. The classical psychedelic, psilocybin, induces long-lasting improvement of cognitive flexibility, but widespread use is infeasible due to legislative restrictions and undesirable non-therapeutic effects. Ideally, the therapeutic pro-cognitive effects of psychedelics could be dissociated from the psychedelic trip, though this requires investigation as to the mechanisms of psychedelic cognitive effects. Cognition, and cognitive flexibility, is achieved through cortical networks: frontal cortically-directed coactivated cortical regions that engage in cooperative processing to meet cognitive demands. Understanding how psychedelics impact neural circuits underlying cortical network regulation is necessary for the development of therapeutics that reproduce the pro-cognitive psychedelic effect. The claustrum, a subcortical nucleus, connects frontal cortical and parietal cortical network nodes via "cortico-claustro-cortical circuits", is required for optimal task performance in cognitively demanding tasks over nondemanding tasks, and is activated at the emergence of a task-positive cortical networks in response to a difficult cognitive task. The claustrum expresses serotonin receptors targeted by psilocin, the active metabolite of psilocybin (5-HTR2a,1d, and 1b) and claustrum deactivation during psilocybin administration is associated with psilocybin-mediated cortical network dysfunction. As such the claustrum represents a prime target for investigation of the cognitive effects of psychedelics. Our preliminary data in mice leads to our hypothesis that serotonin signaling acutely suppresses cortico-claustro-cortical circuits by: suppression of frontal cortical input to claustrum, increased local inhibition of claustrum projection neurons, and decreasing excitability of claustrum projection neurons.

Quantifying Neuropsychiatric Symptoms in Post-Acute Sequelae of COVID-19 (PASC) using the NIH Toolbox® and PROMIS

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Post-acute sequelae of COVID-19 (PASC) afflicts 30-80% of COVID-19 survivors. Quantifying neuropsychiatric complaints using standardized assessments may aid in evaluating and treating PASC. 30 PASC (20 women, 21-63 years) and 27 controls (16 women, 25-68 years) completed three batteries from the NIH Toolbox® for Assessment of Neurological and Behavioral Function (NIHTB) and selected tests from the Patient-Reported Outcomes Measurement Information System (PROMIS). Group differences on fully corrected T-scores were evaluated using analysis of covariance and Cohen's *d* effect sizes. A linear regression model predicted the effects from time since diagnosis. On NIHTB-EB, PASC reported poorer psychological well-being compared to controls (d=-1.12, p=1.2x10^{-4}). Despite 79% of PASC endorsing memory problems, no deficits were found on NIHTB-CB (all p>0.22). On NIHTB-MB, relative to controls, PASC were slower on 2-Minute Walk (d=-0.98, p=4.7x10^{-4}), 4-Meter Walk (d=-0.74, p=0.01), and Dominant-Hand Pegboard (d=-0.74, p=0.01), and had a steeper age-related decline on 2-Minute Walk (Age*Group p=0.03). On PROMIS, PASC had higher scores than controls for depression, anxiety, fatigue, and pain, and poorer global mental and physical health (all p<1.9x10^{-4}). Four cognitive and three motor tests improved with longer time since diagnosis (all p<0.04). NIHTB and PROMIS captured the poorer emotional health and motor function in PASC, including novel findings on locomotion and dexterity ~7 months post-infection. The normal cognitive performance suggests subclinical effects that may be compensated by neural and cognitive reserves, and possibly exacerbated by the negative psychological effects and fatigue. The long-lasting effects of emotional and psychiatric symptoms necessitate mental health treatment be prioritized.

Compulsive Alcohol Consumption is Regulated by Dorsal Striatum Fast-Spiking Interneurons

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Compulsive alcohol consumption is a core, treatment-resistant feature of alcohol use disorder. The dorsolateral striatum encodes the habitual action sequences that underly compulsive behavior. Currently little is known of how chronic ethanol exposure modulates the dorsolateral striatum to drive compulsive drinking. We previously demonstrated that Parvalbumin-expressing striatal fast-spiking interneurons (FSIs are necessary for the development of compulsive ethanol consumption. Specifically, we demonstrated that selectively ablating FSIs in adult male and female C57BL/6J mice undergoing a voluntary intermittent ethanol consumption paradigm reduced ethanol consumption and abrogated compulsive consumption of ethanol with the added bitterant quinine. Next, we explored how chronic ethanol exposure dysregulates striatal FSI physiology to promote compulsive drinking. We recorded synaptic transmission onto striatal FSIs and found that chronic ethanol exposure specifically reduces GABAergic transmission, but not glutamatergic transmission. In contrast, acute bath application of ethanol potentiates GABAergic transmission onto FSIs. Notably, in chronically exposed mice, acute bath application of ethanol produces little to no effect on GABAergic transmission, suggesting that repeated exposure induces homeostatic plasticity to blunt the effects of ethanol exposure. Perineuronal nets (PNNs), a subcomponent of the extracellular matrix that are predominantly found on FSIs, stabilize synaptic transmission onto FSIs. Additionally, drugs of abuse, including alcohol, dysregulate perineuronal nets in cortex. Here we demonstrate that chronic ethanol exposure reduces dorsal striatum PNN protein expression and that enzymatically reducing PNNs reduces GABAergic transmission similarly to chronic ethanol exposure. Together these findings point to novel insight into the neurobiological mechanisms underlying compulsive drinking.

Direct Visualization of Triheteromeric NMDA Receptors

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Glutamatergic signaling via NMDA receptors (NMDARs) is critical in synaptic plasticity, brain development, excitotoxicity, and numerous degenerative and cognitive disorders. Each functional NMDAR comprises four subunits: two obligatory GluN1 subunits and two GluN2A-D or GluN3A-B subunits. Importantly, NMDARs with different GluN2 subunit compositions display strikingly different biophysical characteristics, and the GluN2 subunits also control unique protein interactions and signaling. Accordingly, the physiological characteristics of excitatory synaptic transmission and plasticity, as well as synaptic pathology, depend on the NMDAR subtypes expressed and trafficked to synapses. Thus, identifying how neurons control abundance of specific NMDAR subtypes has been a longstanding goal in neuroscience. Unfortunately, our understanding of these mechanisms has been crucially restricted by inability to visualize one of the key classes of NMDARs in neurons, triheteromeric receptors that contain GluN1 and two different GluN2 subunits. Indeed, in many parts of the brain including the hippocampus, triheteromeric NMDARs containing both GluN2A and GluN2B subunits are the most common NMDAR subtype. However, the subcellular distribution of triheteromeric NMDARs remains mysterious and the mechanisms controlling their trafficking to and from synapses remain almost totally unknown. Here, I introduce a new method for direct visualization of triheteromeric NMDARs in neurons using biomolecular complementation. Using this tool, I find that triheteromeric receptors traffic avidly to synapses and display unique subcellular trafficking characteristics. These probes will fill longstanding gaps in our knowledge of NMDARs and lay necessary groundwork for investigation of other aspects of triheteromeric NMDAR trafficking in healthy neurons and disease models.

Cortical surface area and thickness mediate the association between bullying victimization and cognitive performance in preadolescents: a longitudinal study.

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Objective: Bullying victimization is associated with poorer reading, writing, and grammar performance. Two longitudinal brain morphometry studies in children or adolescents identified the fusiform gyrus and putamen as vulnerable regions. No studies have identified how brain regions affected by bullying may mediate the relationship between victimization and cognitive performance.
Methods: We used the Adolescent Brain Cognitive Development Study 3.0 dataset to identify participants with caregiver-reported bullying victimization (N=323) and matched controls (N=323) at the baseline and 3rd annual (2nd follow-up) visits. We evaluated associations between victimization, cognitive performance, subcortical volume, cortical surface area and thickness, as well as whether any identified brain morphometric changes mediated the relationship between victimization and cognition.
Results: Bullied children had lower scores on reading, processing speed, and inhibitory control and attention ($P<0.05$). They also had smaller putamen volumes ($P<0.05$), smaller insula surface area ($P=0.038$), larger surface area in the superior parietal, entorhinal, pars orbitalis, and pericalcarine regions ($P=0.008$-0.029), and thinner cortices in the precentral, banks of superior temporal sulcus (STS), frontal pole, and rostral middle frontal regions ($P=0.008$-0.037). Additionally, larger surface area in the entorhinal and pars orbitalis and thinner cortices in the banks of STS and precentral mediated 4-14% of the relationship between victimization and cognitive performance ($P<0.05$).
Conclusions: Our novel findings of additional brain regions involved, and the significant mediating relationships support our hypothesis that changes in specific brain regions may mediate the relationship between bullying victimization and specific cognitive outcomes. Future studies will assess the role of functional brain connectivity networks.

BDNF signaling in the nucleus accumbens mediates alcohol reward

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The nucleus accumbens is an essential integration center for circuits governing reward learning and motivated behavior. Plastic changes at synapses onto nucleus accumbens medium spiny neurons enable reward learning. While nucleus accumbens medium spiny neurons receive both excitatory and inhibitory input to regulate their output, very little is known about the mechanisms underlying plastic changes at inhibitory synapses and how this may be modulated by alcohol to hijack the reward system. At these medium spiny neuron inhibitory synapses, we previously discovered a tropomyosin kinase B (TrkB) receptor-mediated long term synaptic depression in the nucleus accumbens core (NAcc-iLTD) that is augmented by acute ethanol exposure. To investigate the behavioral implications of these findings, we here induce NAcc-iLTD in vivo and discover that it results in a conditioned place preference dependent upon BDNF/TrkB signaling. Moreover, subthreshold induction of NAcc-iLTD together with a subthreshold dose of ethanol drives a conditioned place preference. Taken together, our data demonstrate that BDNF/TrkB signaling regulates inhibitory synaptic plasticity in the NAcc and suggest that ethanol disinhibits the NAcc through this mechanism to, at least in part, modulate alcohol reward.

Sex-specific effects of prenatal tobacco exposure on cognitive and brain morphometry measures in preadolescent children

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Prenatal tobacco exposure (PTE) affects cognitive and brain developmental outcomes. However, specific brain regions and sex-specific effects associated with PTE are not well defined. We used baseline data from 9 and 10-year-old children with (n=620) and without (n=10,989) PTE, who are participating in the Adolescent Brain, Cognitive Development study. We obtained parent-reported prenatal exposures to tobacco, alcohol, and marijuana. We also obtained structural MRI (FreeSurfer-processed) and neurocognitive (NIH Toolbox®) measures. Linear mixed models were used to compare groups and assess possible sex-specific effects associated with PTE, while covarying for socio-demographics, hemisphere, and prenatal exposures to alcohol and marijuana as fixed effects, and ABCD sites as random effects. We used false-discovery rate to correct for multiple comparisons, with significance at corrected-$p < 0.05$. PTE-children had poorer performance (corrected-$p \leq 0.01$) than unexposed children on executive function, working memory, episodic memory, reading, crystallized intelligence, fluid intelligence and overall cognition. Compared to unexposed children, PTE-children also had thinner parahippocampal cortices, smaller cortical surface areas (posterior-cingulate gyrus, pericalcarine, lingual and inferior parietal gyri) and smaller subcortical volumes (thalamus, globus pallidum and nucleus accumbens, all corrected-$p \leq 0.001$). Furthermore, only PTE-girls had smaller surface areas in the superior-frontal (interaction-$p=0.03$), precuneus (interaction-$p=0.02$) and postcentral gyrus (interaction-$p=0.01$), while only PTE-boys had smaller putamens (interaction-$p=0.02$). Our findings suggest PTE may lead to lowered cognitive performance and possible delay in brain structures, in a sex-specific manner, in pre-adolescent children.

The Role of Calcitonin Gene-Related Peptide in the Insular Cortex

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Despite its prevalence, chronic pain is largely resistant to therapy. Treatments targeting aversive-affective processing of pain, centered around the parabrachial nucleus (PB), may prove a promising alternative. Calcitonin gene-related peptide (CGRP)-expressing PB neurons densely project to the agranular insula, a brain region shown to be hyperactive in chronic pain. We are testing the overarching hypothesis that this CGRP-expressing PB projection to the insula is causally involved in driving the affective component of chronic pain. The first aim of this project is to test whether CGRP release from PB potentiates post-synaptic responses in insular neurons, as we have previously shown in the amygdala. To test this, we performed whole-cell, voltage-clamp recordings from slices of mouse insula and tested the effect of either bath application or optogenetically-released CGRP. Neither manipulation affected the amplitude or frequency of synaptic inputs to insula neurons, suggesting CGRP does not have a direct effect on insula neurons. RNAscope established that CGRP receptor expression is restricted to glia and vascular endothelium. However, in insula neurons from animals with persistent pain there is an increased occurrence of extrasynaptic, slow transient currents. These are thought to arise from astrocytic glutamate release, suggesting an upregulation in glial activation or receptor expression in the insula in chronic pain. We are now testing the alternative hypothesis that CGRP affects insula neuronal function indirectly via glial/vascular intermediaries. *This work was supported by National Institutes of Health National Institute of Neurological Disorders and Stroke Grants R01NS099245 and R01NS069568.*