Poster Abstracts

1. **Adler, Joy**
   CU Anschutz School of Medicine
   Dopamine D2 receptor modulation of NMDA receptor signaling in the nucleus accumbens
   Dopamine D2-receptors control neural circuits throughout the brain by modulating synaptic inputs and regulating synaptic plasticity, and their dysfunction has been implicated in multiple psychiatric disorders. However, the cellular and synaptic mechanisms by which D2 receptors directly influence postsynaptic glutamate receptors remains poorly understood. Using ex-vivo slice electrophysiology in combination with optogenetics and iontophoresis, we found D2 receptors of the nucleus accumbens inhibit NMDA receptors via a putative direct interaction with GluN2B containing subunits, which was independent of canonical intracellular second messenger pathways. This modulation occurred in a synapse-specific manner, which was set by the amount of glutamate spillover, and could be potentiated following chronic administration of cocaine. We propose that NMDAR modulation by D2 receptors in the NAc occurs through non-canonical, direct dimerization between these two receptors, which function to filter signaling of glutamatergic inputs to the accumbens.

2. **Asbell, Quinn**
   CU Anschutz School of Medicine
   Fzd7: Potential Regulator of Activity-Dependent Astrocyte Morphology
   Astrocytes, distinguished by their star-like morphology, establish connections with neurons, synapses, vasculature, microglia and oligodendrocytes. These interactions underpin functions in development, health and disease. Most of the previous research in glial cell biology has centered on cell-cell interactions of astrocytes, while how these cell-cell interactions are encoded at the genomic level are largely unknown. In our lab, we focus on epigenetic regulation in astrocytes, specifically histone serotonylation, a process of serotonin binding to histones and altering gene

regulation. Previously, we found that mutant astrocytes, unable to undergo histone serotonylation, have altered morphology in the mouse olfactory bulb. Since cellular morphology is a key regulator of cell-cell interactions, we asked what histone serotonylated genes are responsible for astrocyte morphology. In the olfactory bulb, odor exposure results in altered gene expression, as observed through histone serotonylation ChIP and ATAC sequencing data. Reviewing this epigenetic data, a list of genes that have potential roles in morphology was generated. Among these, the gene Frizzled 7 (Fzd7) is highly expressed in astrocytes. We further showed that the astrocytic protein expression level of Fzd7 is increased in mice exposed to odor, potentially pointing to the activity-dependent nature of Fzd7 induction in olfactory bulb astrocytes. Based on these observations, we hypothesize that Fzd7 is an activity-dependent regulator of astrocyte morphology. This will be tested through characterization of a Fzd7 knockout and overexpression model. Using these mouse models, we will investigate neuron-astrocyte interactions through cellular morphology (Aim 1), paired with odor behavioral testing and analyses of synapses (Aim 2). Finally, we will also characterize serotonin's role in regulating odor-evoked astrocyte morphology (Aim 3). Together, these studies will uncover how astrocyte epigenetic changes regulate cell-cell interactions with neurons and ultimately influence behavior.

3. Banh, Jenna

CU Anschutz School of Medicine

A headfixed strain gauge mechanism for studying visually evoked body movement in mice

Studying the neural basis of sensorimotor processing is particularly powerful in headfixed mouse models, though a key limitation is the mouse's inability to move freely. To address this, we developed a strain gauge system to study the way brain activity and cellular populations are involved in visually evoked orienting behaviors, including eye movements and body movements. Custom components and designs of the strain gauge were developed in collaboration with the Neural Engineering Core within the NeuroTechnology Center. A head-fixed mouse was placed in a custom FDM 3D-printed tube, allowing exertion of forces relative to a custom headbar rigidly held (Thorlabs). These forces were transmitted through additional custom 3D-printed interfaces and measured using three off-the-shelf load cells (Wheatstone Bridge, SparkFun TAL221) arranged to capture forces along three axes.Wheatstone Bridges are typically designed for unidirectional force detection and thus custom circuitry was implemented to rail-split the Wheatstone Bridge, enabling bidirectional (including vibrational) force detection. The analog signals were digitized, calibrated, and validated against synchronized video recordings. This system achieves high sensitivity, detecting forces as subtle as tongue contacts with water droplets, without introducing measurable electrical noise. With this design, we are able to record the mouse's eye as it attempts to move its body. Preliminary experiments with this model begin to reveal how body movements are coordinated with eye movements. Computational analyses allow us to further explore the temporal relationship between body movements and saccadic events, and how manipulating cell populations can affect this relationship. These pilot findings highlight how the strain gauge system is a powerful model for understanding key elements of orienting behavior that are often missed in traditional head-fixed experiments. For another study, we use the strain gauge to measure attempted escape movements in response to a looming stimulus. We developed a visual stimulus meant to mimic an incoming predator. In response to this stimulus, a

mouse will attempt to escape the perceived threat, and the force generated by this attempt can be translated into signals that can be analyzed using a variety of methods. Now that this escape behavior can be repeated and measured, we have the ability to manipulate the brain's activity and measure how these manipulations affect behavior. In one case, we use muscimol, an agonist for the GABA receptor, that will inhibit neural activity. For this study, we deliver muscimol to the superior colliculus via a cannula that is cemented in the skull at the time of head fixing surgery. This cannula can then be used to repeatedly access the deep layers of the superior colliculus over a series of recordings. Another method of studying the role of superior colliculus in visually evoked escape responses is to use a viral injection that will target specific cell populations that may be involved in this behavior. Our current plans are to target a class of glutamatergic cells that express the PitX2 transcription factor. By reversibly inhibiting this population of cells, we can begin to explore how PitX2 expressing neurons may be involved in initiating orienting movements required for escape.

4. **Chang, Victoria**
   CU Anschutz School of Medicine
   Role of 5-HT1A Receptors in Excitatory Synaptic Weakening in the PFC
   Serotonin (5-HT) plays a crucial role in the proper development of the brain with disruptions in gestational and early postnatal 5-HT levels having been shown to result in behavioral impairments associated with neurodevelopmental disorders such as autism spectrum disorders (ASDs). The prefrontal cortex (PFC) mediates several of the cognitive and emotional processes that are often disrupted in these disorders and importantly, is densely innervated by serotonergic projections. Several lines of evidence suggest that 5-HT plays a crucial role in the proper development of excitatory synapses within the PFC. However, the mechanisms through which 5- HT influences synapse maturation is still being uncovered. 5-HT acts through several distinct receptor types, one being the inhibitory 5-HT1A receptor (5-HT1AR). Disruption of activity at the 5-HT1AR has been implicated in several neurodevelopmental and neuropsychiatric disorders and it has been identified as being crucial for normal adult behavior in mice. Furthermore, activation of 5-HT1ARs can inhibit the induction of long-term synaptic potentiation, indicating a potential role of 5-HT1ARs in synaptic plasticity. However, despite its developmental importance, the mechanisms through which 5-HT1ARs regulate excitatory synapse structure and function in the developing PFC are yet to be elucidated. Initial findings show that in the developing PFC, 5-HT1AR activation results in excitatory synapse weakening and elimination in a glutamatergic-activity independent manner, further supporting the importance of the 5-HT1AR in early synapse development. Here, we aim to better understand the role of 5-HT1ARs in synaptic and circuit maturation in the PFC, and the impact of its activation during early brain development.

5. **Correa, Vanessa**
   Colorado State University
   The autism-associated loss of δ-catenin functions disrupts social behavior
   δ-catenin is expressed in excitatory synapses and functions as an anchor for the glutamatergic AMPA receptor (AMPAR) GluA2 subunit in the postsynaptic density. The glycine 34 to serine

(G34S) mutation in the δ-catenin gene has been found in autism spectrum disorder (ASD) patients and results in loss of δ-catenin functions at excitatory synapses, which is presumed to underlie ASD pathogenesis in humans. However, how the G34S mutation causes loss of δ-catenin functions to induce ASD remains unclear. Here, using neuroblastoma cells, we discover that the G34S mutation increases glycogen synthase kinase 3β (GSK3β)-dependent δ-catenin degradation to reduce δ-catenin levels, which likely contributes to the loss of δ-catenin functions. Synaptic δ-catenin and GluA2 levels in the cortex are significantly decreased in mice harboring the δ-catenin G34S mutation. The G34S mutation increases glutamatergic activity in cortical excitatory neurons while it is decreased in inhibitory interneurons, indicating changes in cellular excitation and inhibition. δ-catenin G34S mutant mice also exhibit social dysfunction, a common feature of ASD. Most importantly, pharmacological inhibition of GSK3β activity reverses the G34S-induced loss of δ-catenin function effects in cells and mice. Finally, using δ-catenin knockout mice, we confirm that δ-catenin is required for GSK3β inhibition-induced restoration of normal social behavior in δ-catenin G34S mutant animals. Taken together, we reveal that the loss of δ-catenin functions arising from the ASD-associated G34S mutation induces social dysfunction via alterations in glutamatergic activity and that GSK3β inhibition can reverse δ-catenin G34S-induced synaptic and behavioral deficits.

6. Dabertrand, Fabrice

CU Anschutz School of Medicine

Pericyte KATP Channel Hyperactivity disrupt Cortical Perfusion Equalization in Cerebral Small Vessel Disease: Evidence from three-photon imaging

Background: Cerebral small vessel diseases (SVDs), including the genetic form CADASIL, disrupt brain microcirculation and contribute to vascular dementia and stroke. CADASIL, caused by NOTCH3 mutations, leads to receptor accumulation and extracellular matrix aggregation, with no current treatment. While its pathology has been studied in various cell types, pericytes, key regulators of capillary function, remain understudied despite high NOTCH3 expression. Single-cell RNA-seq has identified Kir6.1, a KATP channel subunit, as highly expressed in pericytes and critical for cerebral blood flow regulation. We thus investigated Kir6.1 in CADASIL to uncover potential functional changes. Study objectives: To explore the role of pericytes and capillaries in CADASIL, we adopted a multimodal investigative strategy. This included molecular analyses alongside both ex vivo and in vivo techniques to examine their impact on myogenic tone, vasoreactivity, and the regulation of cerebral blood flow at the capillary level. Methods: To examine CADASIL-related changes in pericyte and capillary function, we used TgNotch3R169C mice and wild-type controls. Spatial transcriptomics (10x Genomics Visium) on brain sections and isolated microvasculature revealed gene expression differences. Ex vivo assays, including pressure myography and patch-clamp electrophysiology, assessed KATP channel activity. In vivo, two-photon microscopy following cranial window implantation measured microvascular dynamics. In silico modeling evaluated cortical perfusion, which was validated using three-photon microscopy to visualize blood flux distribution. Results: Our 10X Genomics Visium Spatial Transcriptomics revealed metabolic abnormalities in CADASIL brain slices and isolated microvasculature. Notable pathways include differential ATP synthesis and mitochondrial transport. In both in vivo and ex vivo experiments, myogenic tone was significantly decreased at the capillary level. Given the Kir6.1

channel's role in regulating capillary blood flow, we then investigated its potential contribution to the decreased tone. Our pressure myography and in vivo results show that CADASIL capillaries had a minimal dilation to KATP agonist pinacidil (17% vs. 44%) and a robust vasoconstriction to antagonist glibenclamide (28% vs. 10%) compared to controls. Further probing KATP channel hyperactivity in CADASIL capillaries, we found that pericytes exhibited a significantly higher inward basal current, which was sensitive to the KATP antagonist glibenclamide. In silico modeling using our KATP channel hyperactivity findings revealed increased blood flow in the superior cortical layers at the expense of layer V and VI, leading to significant hypoperfusion in this region. This prediction was confirmed with three-photon imaging, which allowed direct quantification of red blood cell flux across cortical layers. Conclusions: Our findings reveal that CADASIL-associated KATP channel hyperactivity in pericytes disrupts capillary tone and responsiveness, contributing to cortical layer-specific hypoperfusion. These results highlight a previously underappreciated role of pericyte dysfunction in CADASIL pathophysiology and suggest that targeting Kir6.1 may offer a novel therapeutic avenue for restoring microvascular regulation and improving cerebral perfusion. The incorporation of three-photon imaging provides a powerful tool to study microcirculatory dynamics in deeper cortical structures, enhancing our ability to validate in silico models and interpret disease impact across cortical layers.

7. Ellor, Sarah

CU Anschutz School of Medicine

Neuronal activity-dependent induction of astrocytic Sox7 in sensory processing

Astrocytes are the largest population of non-neuronal cells in the brain and interact with neurons to functionally modulate behavior. It has been previously shown that, in the olfactory bulb, astrocytes are necessary for the processing of olfactory stimuli. While we know that astrocyte-neuron interactions are necessary for sense of smell, the molecular mechanisms by which astrocytes regulate olfaction are unknown. Using RNA-Seq in an astrocyte reporter mouse line Aldh1l1-GFP, we identified astrocyte-specific GFP+ differentially expressed genes in the olfactory bulb that are induced in response to odor stimulation. Among these genes we found the transcription factor Sox7 as significantly upregulated. We further validated that Sox7 protein levels are increased in GFP+ astrocytes after odor stimulation in comparison to controls with oil exposure. Since Sox7 gene expression was upregulated in a relatively short amount of time after odor exposure, we next asked if Sox7 shares characteristics of immediate-early genes (IEGs), which are known for rapidly initiating cellular cascades in response to external stimuli. We show that in the olfactory bulb of odor-stimulated mice, Sox7 protein expression is rapidly increased in astrocytes at the same timepoint as neuronal Fos, a known marker for neuronal activity. Furthermore, using variable time-points of odor stimulation, we show that astrocytic Sox7 induction is transient and dynamic, comparable to well-established IEGs. Excitingly, we found odor-evoked Sox7 induction to be exclusively in astrocytes in the olfactory bulb. This leads to the hypothesis that astrocytic Sox7 transcription factor mediates astrocyte-neuron interactions in olfactory processing. To test this hypothesis, we are generating Sox7 overexpression and Sox7 conditional knockout mouse models to investigate astrocytic Sox7 function in olfactory processing. Taken together, our studies will uncover how sensory processing is modulated by molecular regulators, such as potential astrocytic IEGs that are induced in response to sensory stimulation.

8. **Elsayed, Nada**
Vanderbilt University
Exploring single cell alterations during forebrain development in an organoid model of tuberous sclerosis complex.
Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder caused by mutations in the TSC1 or TSC2 genes. Resultant overactive mTOR signaling results in unregulated cell growth, manifesting as dysplastic cortical brain lesions called tubers. Tubers are associated with debilitating epilepsy throughout life, often necessitating surgical removal, but the molecular mechanisms underlying their development remain largely unclear. In this study, we sought to understand protein-level changes in cellular identity during dorsal- or ventral-directed cortical differentiation of TSC2-mutant cells. Three isogenic sets of patient-derived induced pluripotent stem cells (iPSCs) were generated with heterozygous, homozygous, and corrected mutations in TSC2. iPSCs were differentiated into dorsal or ventral forebrain organoids and collected at defined intervals for CyTOF analysis. Our custom built 40-antibody panel encompassed markers of neural lineage and phosphoproteins downstream of mTOR, EGFR, and MAPK signaling pathways. Across isogenic lines, TSC2-/- neural progenitor cells exhibited a distinct protein phenotype as early as ten days following neural induction, including elevated SOX2 and YAP1 expression coinciding with mTORC1-specific phosphorylation. CyTOF analysis of surgically resected tubers from patients with TSC also showed abnormal cell signatures. Immunostaining of early dorsal TSC2-/- organoids revealed unexpected expression of markers typically associated with ventral structures. These results indicate a broader level of identity dysregulation than previously reported. Functional neuronal data using multi-electrode arrays also reveal premature firing and electrical exhaustion in TSC2-/- neurons. Collectively, these data suggest that loss of TSC2 leads to altered dorsal and ventral cell fate and neuronal function. Future work will assess the impact of targeted pharmacological intervention on ameliorating identified phenotypes.

9. **Fisher, Natalie**
CU Anschutz School of Medicine
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Studying the neural basis of sensorimotor processing is particularly powerful in headfixed mouse models, though a key limitation is the mouse's inability to move freely. To address this, we developed a strain gauge system to study the way brain activity and cellular populations are involved in visually evoked orienting behaviors, including eye movements and body movements. Custom components and designs of the strain gauge were developed in collaboration with the Neural Engineering Core within the NeuroTechnology Center. A head-fixed mouse was placed in a custom FDM 3D-printed tube, allowing exertion of forces relative to a custom headbar rigidly held (Thorlabs). These forces were transmitted through additional custom 3D-printed interfaces and measured using three off-the-shelf load cells (Wheatstone Bridge, SparkFun TAL221) arranged to capture forces along three axes.Wheatstone Bridges are typically designed for unidirectional force detection and thus custom circuitry was implemented to rail-split the Wheatstone Bridge, enabling bidirectional (including vibrational) force detection. The analog signals were digitized, calibrated, and validated against synchronized video recordings. This system achieves high

sensitivity, detecting forces as subtle as tongue contacts with water droplets, without introducing measurable electrical noise. With this design, we are able to record the mouse's eye as it attempts to move its body. Preliminary experiments with this model begin to reveal how body movements are coordinated with eye movements. Computational analyses allow us to further explore the temporal relationship between body movements and saccadic events, and how manipulating cell populations can affect this relationship. These pilot findings highlight how the strain gauge system is a powerful model for understanding key elements of orienting behavior that are often missed in traditional head-fixed experiments. For another study, we use the strain gauge to measure attempted escape movements in response to a looming stimulus. We developed a visual stimulus meant to mimic an incoming predator. In response to this stimulus, a mouse will attempt to escape the perceived threat, and the force generated by this attempt can be translated into signals that can be analyzed using a variety of methods. Now that this escape behavior can be repeated and measured, we have the ability to manipulate the brain's activity and measure how these manipulations affect behavior. In one case, we use muscimol, an agonist for the GABA receptor, that will inhibit neural activity. For this study, we deliver muscimol to the superior colliculus via a cannula that is cemented in the skull at the time of head fixing surgery. This cannula can then be used to repeatedly access the deep layers of the superior colliculus over a series of recordings. Another method of studying the role of superior colliculus in visually evoked escape responses is to use a viral injection that will target specific cell populations that may be involved in this behavior. Our current plans are to target a class of glutamatergic cells that express the PitX2 transcription factor. By reversibly inhibiting this population of cells, we can begin to explore how PitX2 expressing neurons may be involved in initiating orienting movements required for escape.

10. **Futia, Gregory**

CU Anschutz Bioengineering

All optical neural recording and cell-specific patterned optogenetic stimulation in a freely moving mouse using a MEMS based two-photon miniscope (Opto2P-FCM)

Multiphoton microscopy combined with optogenetic photostimulation is a powerful technique in neuroscience enabling precise control of cellular activity to determine the neural basis of behavior in a live animal. Two-photon photostimulation has taken this further by allowing interrogation at the individual neuron level. However, it remains a challenge to implement imaging of neural activity with cell-specific patterned two-photon photostimulation in a freely moving mouse. We developed a miniature microscope for high resolution two-photon fluorescence imaging with spatially patterned two-photon optogenetic stimulation. The design incorporates a MEMS scanner for two-photon imaging and a second beam path for patterned two-photon excitation in a compact and lightweight design that can be head-attached to a freely moving animal. We demonstrate spatially localized optogenetics and high resolution MEMS based two-photon imaging in a freely moving mouse. The new capabilities of this miniature microscope design can enable cell-specific studies of behavior that can only be done in freely moving animals.

11. **Gomez Wulschner, Luis**
CU Anschutz School of Medicine
Nicotinic signaling drives silent synapse maturation in the developing prefrontal cortex
Layer 2/3 pyramidal neurons in the developing prefrontal cortex (PFC) contain immature silent spines, key substrates for experience-dependent circuit formation. The cholinergic system modulates synapse development, but its role in silent spine maturation remains unclear. We show that ablation of PFC-projecting cholinergic fibers during early mouse cortical development delays silent spine unsilencing, whereas acetylcholine or nicotine treatment of mouse PFC slices facilitates their functional maturation. Using two-color, two-photon uncaging of nicotine and glutamate, we demonstrate that activation of postsynaptic nicotinic acetylcholine receptors (nAChRs) at individual silent spines induces unsilencing via α7 and α4β2 receptors and Protein Kinase A (PKA) signaling, independent of glutamatergic activity. Perinatal nicotine exposure accelerates silent spine maturation, highlighting the sensitivity of developing excitatory synapses to cholinergic modulation. Together, these findings establish nAChR signaling as a critical regulator of excitatory synapse maturation and reveal a mechanistic link by which cholinergic input shapes prefrontal circuit assembly. This work provides a framework for understanding how early-life cholinergic perturbations, including nicotine exposure, influence synaptic maturation and may contribute to neurodevelopmental disorders involving altered PFC function, with potential implications for cognition and behavioral outcomes.

12. **Hickman, Jordan**
CU Anschutz School of Medicine
Neural population dynamics of direct electrical stimulation of mouse primary visual cortex
Intracranial electrical stimulation underpins neuromodulation and cortical prostheses, yet how a single pulse engages neural tissue and integrated into perception remains unclear. Using three orthogonal Neuropixels probes in mouse visual areas, we recorded extracellular voltages and spikes with sub-millisecond resolution in 3D around a stimulation source. The evoked potential extended sub-linearly with amplitude and its shape asymmetrically influenced by anatomical heterogeneity. Increasing current increased the density, but not spatial extent, of directly responsive neurons, but fewer than 5% of neurons within the evoked potential were activated. Fast-spiking interneurons were recruited nearer the source, whereas pyramidal neurons showed anisotropic activation. Direct responses were polarity- and amplitude-selective. Sparse direct spiking drove large, asymmetric cortical responses and modulated >50% of neurons at higher amplitudes. Mice weakly detected single pulses, and performance did not improve with amplitude. Thus, anatomy and spatiotemporal patterning—not spike counts—govern perceptual impact, informing biomimetic, multi-contact prostheses.

13. **Hua, Isabelle**
CU Anschutz School of Medicine
Monitoring plasticity-associated dendritic calcium dynamics during learning
Calcium is a signaling molecule essential for synaptic plasticity mechanisms underlying learning and memory. Intracellular calcium release, from the endoplasmic reticulum, has been shown to prominently shape synaptic plasticity. However, the release dynamics of this key intracellular calcium store are unexplored in mammalian neurons in vivo. Here, I leveraged spatially tuned receptive fields known as 'place fields' in hippocampal CA1 pyramidal neurons to investigate when the ER releases plasticity-promoting calcium during learning. Through a combination of imaging and behavioral innovations, I am working to simultaneously capture cytosolic and endoplasmic reticular calcium dynamics in dendrites of single neurons as new place fields form.

14. **Janarthanan, Sanjeev**
CU Anschutz School of Medicine
A Biophysical Model of GCaMP For Improved Spike Inference
Calcium imaging with genetically encoded fluorescent indicators is a key method for monitoring neuronal population activity in vivo. While numerous algorithms exist for spike inference, accurately reconstructing spike trains at firing rates above 20 Hz has remained a challenge. Recent advances, such as the particle Gibbs with ancestor sampling (PGAS) approach (Diana et al., eLife 2024), have improved inference with a Bayesian framework, but rely on linear autoregressive models that fail to capture the nonlinear dynamics of fast GCaMP variants. To address this limitation, we have extended the PGAS framework with a biophysical GCaMP8f model (BiophysPGAS) that incorporates nonlinear fluorescence dynamics (Broussard et al., in preparation). This approach increases the accuracy of spike time predictions but remains computationally intensive. Supervised machine-learning methods such as CASCADE (Rupprecht et al., Nat. Neurosci. 2021) provide rapid inference and lower accuracy but depend on large, high-quality training datasets that are difficult to obtain. We introduce an end-to-end spike inference pipeline that combines the strengths of these approaches. We benchmarked our algorithm on experimental calcium imaging data with simultaneous recordings of ground-truth spikes, as well as on calcium buffering model simulations that represent different cell types. First, PGAS is used to infer spike times and extract cell-specific parameters from experimental recordings. These parameters then generate synthetic training datasets, which are used to train supervised inference methods. This hybrid strategy enables accurate, efficient spike reconstruction while reducing reliance on extensive ground-truth data.

15. **Karas, Stephanie**
CU Anschutz School of Medicine
The Role of Perivascular and Meningeal Fibroblast Calcium Signaling in Vasodynamics and Cerebrovascular Health
Alzheimer's disease (AD) is often characterized by cerebral amyloid angiopathy (CAA), in which amyloid-beta (Aβ), a waste byproduct normally cleared by cerebrospinal fluid (CSF), accumulates

along the walls of leptomeningeal arteries. This buildup compromises vessel integrity and disrupts vasomotion - the rhythmic constriction and dilation of blood vessels that facilitates blood flow. Disruption of vasomotion impairs CSF movement through the brain, and thereby, proper waste clearance, contributing to further Aβ accumulation and disease progression. Perivascular cells, such as smooth muscle cells and perivascular macrophages, are known to regulate vessel dynamics. We recently discovered that perivascular fibroblasts (PVFs) can influence vasodynamics via calcium signaling, potentially in conjunction with smooth muscle cell crosstalk. However, the role of meningeal fibroblasts found in close proximity to these vessels is not fully understood. This study focuses on the dynamics of calcium signaling in perivascular and meningeal fibroblasts and their relationship to vasomotion under normal conditions, with the goal of understanding how these mechanisms might be altered in the presence of disease. We hypothesize that meningeal fibroblast calcium dynamics are coordinated with vessel movement and play a role in maintaining vascular function and waste clearance, which could become disrupted in the context of Aβ pathology. We generated fibroblast calcium reporter animals by crossing Col1a2-CreER and Ai95flox mice to drive GCaMP6f expression in fibroblasts. Using in vivo two-photon imaging on awake mice, we recorded calcium signaling in perivascular and meningeal fibroblasts alongside vasomotor dynamics in adjacent vessels. We used MATLAB to extract quantitative metrics, including mean frequency and bandpower of calcium signaling and vasomotor activity, and to perform cross-correlation analyses to assess temporal relationships and coordination between calcium signals and vessel diameter changes. Cross-correlation analysis indicated temporally distinct patterns of synchronization between perivascular fibroblast calcium signals and vessel diameter changes, exhibiting two main subgroups: one positively correlated with vasomotion, peaking slightly after diameter change, and another negatively correlated with vasomotion, peaking just before diameter change. Meningeal fibroblasts also showed functional subpopulations: positively correlated cells tightly entrained with vasomotion, and negatively correlated cells displaying symmetrical peaks in activity before and after vessel diameter change, suggesting a potential buffering role. These findings indicate that fibroblasts may support vasodynamics through time-locked signaling patterns. As vascular dysfunction often precedes the symptomatic onset of cognitive impairment by decades, studying how fibroblast signaling dysfunction may contribute to vascular breakdown within AD and CAA disease models could reveal fibroblasts as a potential therapeutic target for preserving CSF flow and waste clearance in the brain. By bridging a gap in the existing knowledge surrounding meningeal fibroblast contributions to vasodynamics, this work offers a new lens through which to examine cerebrovascular health and highlights fibroblast calcium signaling as a promising avenue for future exploration in neurodegenerative disease.

16. Larsen, Matthew

CU Anschutz School of Medicine

APP as a mediator of amyloid-β effects on CaMKII synaptic functions

The proper functioning of learning, memory, and cognition requires the activity-dependent strengthening of excitatory synapses in the hippocampus via a process known as long-term potentiation (LTP). LTP can be impaired in ex vivo hippocampal slices by incubation with the peptide amyloid-β (Aβ)1; increased concentrations of this peptide are highly associated with early

synaptic deficits in Alzheimer's disease (AD), a progressive neurodegenerative disease2. LTP is known to require the Ca2+/calmodulin-dependent protein kinase II (CaMKII), and specifically its localization to excitatory synapses, driven by direct binding of CaMKII to the NMDA-type glutamate receptor GluN2B3. This localization of CaMKII to excitatory synapses is impaired by incubation with Aβ, revealing a potential mechanism underlying Aβ-induced synaptic deficits. Interestingly, the impairments of LTP4 and CaMKII movement caused by exogenous Aβ incubation are alleviated by loss of the amyloid precursor protein (APP). While the proteolytic cleavage that APP undergoes to form Aβ is well-characterized, this apparent downstream role as a mediator of Aβ-induced impairment remains largely unexplored. Importantly, individuals with Down syndrome (DS), a genetic developmental disorder, express increased levels of APP due to triplication of the APP gene5. As loss of endogenous APP "desensitizes" neurons to the synaptic deficits caused by Aβ, it may conversely be true that these increased APP levels "sensitize" neurons to the effects of Aβ. Initial results indicate that APP is not only necessary to mediate CaMKII impairments caused by exogenous Aβ, but also sufficient to impair CaMKII-GluN2B binding in heterologous cells, further implicating APP as a direct mediator of downstream Aβ-induced CaMKII impairments.

17. **Lee, Brent**

CU Anschutz Anesthesiology

Comparison of Unique Ultrastructural Characteristics of Pial and Perivascular Fibroblasts - Implications for Cerebral Small Vessel Disease

The unique characteristics of perivascular fibroblasts (PVFs) in brain vasculature may contribute to their ability of maintaining vessel homeostasis within the brain. Further, we find changes in PVF populations in Alzheimer's Disease (AD) mouse models pointing to their potential contribution to small vessel disease commonly observed in AD. Some characteristics of perivascular fibroblasts have been explored using electron microscopy of mice brains, and while some initial patterns can be drawn, little has been explored regarding characteristics of fibroblasts residing near the pial surface of the brain. To identify unique ultrastructural features of PVFs and pial fibroblasts, we sought out to compare characteristics between PVFs and pial fibroblasts in the MICrONs 1 cubic millimeter 3D electron microscopy dataset from the visual cortex of a healthy mouse brain. We explored differences in fibroblast macrophage interactions, vesicle exchange, vesicle density, proximity to arterioles or venules, and presence of fibroblast "projections" into surrounding tissue. By exploring the potential similarities or differences between pial and perivascular fibroblasts, we aim to discern unique qualities of pial fibroblasts and PVFs that may help explain their role in physiological and pathological situations, and how improper function may contribute to small vessel disease. We show that pial fibroblasts exhibit a lower incidence of fibroblast-macrophage interactions compared to counterparts seen in perivascular spaces. A part of these interactions includes exchange of vesicles between fibroblasts and macrophages, and while a large majority of perivascular fibroblasts demonstrate vesicular exchange between fibroblasts and macrophages, only a small portion of pial fibroblasts demonstrate this action. Furthermore, a phenomenon currently described as "fibroblast projections" is seen within select perivascular fibroblasts but is largely unseen in pial fibroblasts. Based off our initial findings, we observe different characteristics between PVFs and pial fibroblasts indicating differences in roles for maintaining vascular homeostasis and progression of cerebral amyloid angiopathy (CAA).

18. **Lee, Rahmi**
Colorado State University
Co-activation of selective nicotinic acetylcholine receptors is required to restore normal hippocampal rhythmic activity and memory in Alzheimer's disease
Alzheimer's disease (AD) is the most common form of dementia with no known cause and cure. Research suggests that a reduction of GABAergic inhibitory interneurons' activity in the hippocampus by beta-amyloid peptide (Aβ) is a crucial trigger for cognitive impairment in AD via hyperexcitability. Therefore, enhancing hippocampal inhibition is thought to be protective against AD. However, hippocampal inhibitory cells are highly diverse, and these distinct interneuron subtypes differentially regulate hippocampal inhibitory circuits and cognitive processes. Moreover, Aβ unlikely affects all subtypes of inhibitory interneurons in the hippocampus equally. Hence, identifying the affected interneuron subtypes in AD to enhance hippocampal inhibition optimally is conceptually and practically challenging. We have previously found that Aβ selectively binds to two of the three major hippocampal nicotinic acetylcholine receptor (nAChR) subtypes, α7- and α4β2-nAChRs, but not α3β4-nAChRs, and inhibits these two receptors in cultured hippocampal inhibitory interneurons to decrease their activity, leading to hyperexcitation and synaptic dysfunction in excitatory neurons. We have also revealed that co-activation of α7- and α4β2-nAChRs is required to reverse the Aβ-induced adverse effects in hippocampal excitatory neurons. Here, we discover that α7- and α4β2-nAChRs predominantly control the nicotinic cholinergic signaling and neuronal activity in hippocampal parvalbumin-positive (PV+) and somatostatin-positive (SST+) inhibitory interneurons, respectively. Furthermore, we reveal that co-activation of these receptors is necessary to reverse hippocampal network dysfunction and fear memory loss in the amyloid pathology model mice. We thus suggest that co-activation of PV+ and SST+ cells is a novel strategy to reverse hippocampal dysfunction and cognitive decline in AD.

19. **Mahdavi Omaselan Olya. Ali**
CU Anschutz School of Medicine
Heterogeneous synaptic dynamics extend the bandwidth of sensory-motor representations and facilitate temporal learning in a cerebellar cortex model
Dynamic patterns of neural population activity support precise timing of motor behavior. In the cerebellum, granule cells (GCs) are thought to generate high- dimensional spatiotemporal representations of sensory-motor contexts, enabling well-timed associative learning. Yet the mechanisms by which GCs produce such temporally rich dynamics remain incompletely understood. Recent work from our lab showed that heterogeneity in short-term synaptic plasticity (STP) at mossy fiber- granule cell (MF-GC) synapses can endow a feedforward network with temporal structure, enabling the generation of Purkinje cell (PC) pauses in a model of delay eyelid conditioning when MF inputs are static (Barri et al., 2022). Complementary modeling suggests that temporally structured MF activity alone can also support GC dynamics required for learning (Gilmer et al., 2022). However, whether STP heterogeneity enhances learning in the presence of dynamic MF inputs remains unknown. To address this, we estimated trial-averaged MF firing rate dynamics during a forelimb reach-to-grasp task. We used these patterns to drive a two-layer rate-

based perceptron model with either static or STP-modulated synapses. Synaptic weights between GCs and PC were trained using gradient descent. Learning performance was evaluated as a function of the spectral overlap between GC responses and target PC activity. We found that successful learning required GC responses to match or exceed the spectral content of the target signal. Compared to homogeneous or absent STP configurations, diverse STP profiles preserved high temporal sparsity and extended the bandwidth of GC activity. This broadened the range of GC thresholds, yielding accurate learning and reduced output error. In effect, STP heterogeneity increased the robustness and flexibility of the GC basis set. These results demonstrate that combining dynamic MF inputs with heterogeneous STP produces richer GC dynamics, enabling more effective encoding of temporally precise PC activity in tasks such as delay conditioning and reach execution. Our findings provide a mechanistic understanding of how synaptic diversity enables flexible and accurate temporal learning in the cerebellum.

20. McGregor, Match

CU Anschutz School of Medicine

Distinct Spatiotemporal Modes of Dopamine Transmission Drive Motor Output and Motor Learning

Striatal dopamine is critical for executing coordinated movements and motor learning, yet the subcellular mechanisms that drive these functions remain unclear. Within the dorsolateral striatum, sparse dopamine varicosities are thought to signal to postsynaptic receptors through volume transmission. This spatiotemporal mode is characterized by slow changes (>100 ms) in low concentrations of dopamine (< 1 µM) that occur over a broad volume (>10 µm). Recently, emerging evidence suggests that dopamine transmission can also occur through a 'synaptic-like' mode (referred here as focal transmission), which is characterized by fast, localized changes in high concentrations of dopamine. Given dopamine's multiple behavioral functions and the potential existence of two spatiotemporal modes transmission, we test the hypothesis that different spatiotemporal modes of dopamine signaling drive distinct aspects of motor behavior. Using a combination of fast-scan cyclic photometry, slice electrophysiology, and two-photon imaging of fluorescent dopamine sensors, we first show that focal and volume dopamine transmission can be regulated independently of each other. Next, using a battery of manipulations to perturb dopamine release, we demonstrate that while volume transmission is necessary for healthy motor output, focal transmission is sufficient to support motor learning. Lastly, we use a combination of electrophysiology and imaging to examine how these spatiotemporal modes of dopamine transmission shape medium spiny neuron firing and plasticity—functions critical to their behavioral output. Our findings suggest that the spatiotemporal structure of dopamine transmission shapes both its physiological and behavioral function within the dorsolateral striatum.

21. McNulty, Connor

CU Boulder

Active coping evokes distinct corticostriatal response profiles in male and female rats

Coping strategies provide a sense agency when facing adversity and are a common therapeutic target for stress-linked disorders. Learning how one's responses (R) influence outcomes (O) is central to this process; however, the neural basis supporting this form of learning has largely been studied in appetitive contexts. Here, we examine instrumental learning circuitry within the context of adversity, where subjects can control (R) the termination of each stressor exposure (O). Though males and females perform the instrumental controlling response with the same efficacy, recent work suggests that the stress-buffering effects of control are only present in males. EXP1: We used a genetically encoded calcium indicator GCaMP8 to selectively monitor corticostriatal activity during controllable and physically identical uncontrollable stressor. Males exhibited positive corticostriatal responses (AUC and peak normalized dF) with higher dynamic range during both the learning and subsequent maintenance of the controlling response. Females, in contrast, showed reduced corticostriatal activity surrounding coping behavior. EXP2: In a subsequent cohort, the dopamine sensor GRABDA displayed the opposite pattern between the sexes. Optical recordings of the corticostriatal pathway showed strong positive dopamine-mediated fluorescence during R-O events in females, but not males. EXP3: Coping-related photometry findings were supported by fluorescent in situ hybridization of the dopaminergic input to the prefrontal cortex (PFC), the ventral tegmental area (VTA). Females with instrumental control had significantly greater percentages of mesocortical dopamine neurons expressing c-fos mRNA compared to males and other controls. EXP4: Lastly, chemogenetic silencing of the VTA→PFC projection in females led to buffering against the behavioral outcomes of stress exposure. Our findings, using an animal model of coping, suggest sex differences in the circuitry involved in aversively motivated instrumental learning. Notably, corticostriatal differences emerged (1) during R-O associative events and (2) were characterized by an inverse relationship between calcium- and dopamine-mediated fluorescence. Therefore, the circuitry recruited during the coping experience determines its impact, rather than the act of coping itself.

22. Minjarez, Crystal

CU Anschutz School of Medicine

Distinct roles of Pitx2+ and Gad2+ neurons in the superior colliculus in saccades

Survival in dynamic environments depends critically on orienting behaviors. Rapid, coordinated actions, like saccades and head turns, reorient sensory organs towards salient stimuli. The intermediate and deep layers of the superior colliculus (SC) are a crucial conductor in orchestrating these complex sensorimotor transformations. However, the cell-type-specific circuitry underlying the selection and initiation of orienting movements is unclear. We hypothesize that functional inhibition mediates competitive interactions between populations of pre-motor neurons, encoding movements to specific targets. We seek to test this hypothesis by examining how the activity of genetically identified Pitx2+ pre-motor SC output neurons and GABAergic SC neurons relates to saccades. In this study, we expressed DREADDs in Pitx2+ and Gad2+ neurons in separate groups of transgenic mice to silence neuronal activity during a protocol that elicits

frequent saccades. Comparing saccade parameters -- such as start and endpoints, amplitude, duration, and frequency – between test and control sessions, allows us to determine the necessity of Pitx2+ and Gad2+ neurons in normal saccadic behavior. Preliminary results suggest that inhibiting Pitx2+ neurons has little effect on overall saccade frequency but may affect amplitude. Analyses of our Gad+ inhibition data are ongoing; we expect to observe effects on saccades consistent with long-range inhibition within the SC. Together, these experiments aim to uncover cell-type-specific circuit mechanisms underlying precise orienting movements.

23. Munoz, Laura

CU Anschutz School of Medicine

Intercellular and signaling interactions between brain perivascular fibroblasts and macrophages: Implications for vascular homeostasis in health and cerebral amyloid angiopathy

Perivascular spaces (PVS) are fluid-filled compartments that surround blood vessels in the brain and serve as specialized niches for various cell types, including perivascular fibroblasts (PVFs) and perivascular macrophages (PVMs). While the functions of PVMs are increasingly understood, PVFs—a unique population of brain fibroblasts—remain poorly characterized. Given that PVSs are key sites of amyloid-β (Aβ) deposition in cerebral amyloid angiopathy (CAA), which is frequently observed in Alzheimer's disease (AD), PVFs may play an important role in vascular health and be directly impacted by Aβ pathology. In the tgSwDI AD mouse model, which develops early and prominent CAA, we observed a reduction in PVF populations at early disease stages—coinciding with parenchymal Aβ deposition but preceding overt vascular Aβ accumulation. These early PVF losses were accompanied by morphological changes in PVMs, suggesting that disruptions in the PVS niche may precede and potentially contribute to CAA pathology. To better understand PVF–PVM interactions in the healthy brain, we leveraged a large-scale 3D electron microscopy (EM) dataset from the Machine Intelligence from Cortical Networks (MICrONS) program, which provides ultrastructural reconstructions of mouse visual cortex. We identified 303 PVFs and 156 PVMs in a 1 mm^3 EM volume, with PVFs and PVMs being co-localized in over 70–85% of cases. These cells exhibited direct and complex physical interactions, including soma–soma (30%), soma–process (38%), and process–process (32%) contacts. In approximately 78% of PVF–PVM interfaces, we observed shared vesicular structures, with directional vesicle transfer from PVFs to PVMs in the majority (83%) of cases—raising the possibility that PVFs may contribute to vesicle- or lysosome-related processes at the PVS interface. To evaluate whether similar alterations occur in the human brain in the context of AD, we analyzed a publicly available single-cell RNA-sequencing dataset from the human cortex, focusing on PVFs and brain-associated macrophages (BAMs) from both control and AD patients. Transcriptomic profiling revealed disease-associated changes in PVFs related to vesicle/lysosomal processes, particularly in genes involved in autophagosome assembly and maturation, such as VMP1, LAMP1, and LAMP2. Notably, BAMs also showed altered expression of genes including ELMO1, which plays roles in phagocytosis, proinflammatory signaling, and fibrosis. CellChat analysis further revealed a PVF–BAM interaction mediated by the VISFATIN signaling pathway, which is known to regulate immune responses, inflammation, cell proliferation, autophagy, apoptosis, and necrosis. This interaction appeared to be diminished in AD, suggesting impaired crosstalk between these cell types in the disease context. Collectively, these findings indicate a broader disruption of cellular waste clearance mechanisms within the

perivascular niche. Our results support the notion that both PVFs and BAMs are affected in human AD and mouse models, particularly in pathways related to vesicle trafficking and lysosomal function. Altogether, our data suggest that early alterations in PVF–BAM interactions and vesicle dynamics may be key contributors to PVS dysfunction and vascular pathology in AD.

24. Rathan Kumar, Sudiksha

CU Anschutz School of Medicine

Fighting the Fire: HIKESHI-associated leukodystrophy

HIKESHI-associated leukodystrophy (HAL) is a lethal genetic condition with most patients dying in childhood. Patients have hypomyelination associated with developmental delay, paraparesis, and high risk of death following febrile illnesses and infections. HAL is caused by homozygous mutations in the HIKESHI gene leading to loss of protein. HIKESHI serves as a nuclear import carrier to the chaperone HSP70 during heat shock response (HSR). When exposed to physiological stressors, cellular proteins are prone to misfold resulting in dysfunction. In response, cells initiate HSR with chaperone proteins refolding damaged proteins to maintain homeostasis. As the primary symptoms in HAL are related to white matter, we hypothesized that lack of HIKESHI impairs oligodendrocyte HSR resulting in abnormal neural development and function. Skin samples were collected from affected homozygous patients, non-symptomatic heterozygous relatives, and healthy individuals. iPSCs were reprogrammed from primary fibroblast cultures. After validation, iPSCs were differentiated into neurons and oligodendrocytes and exposed to stressors, followed by immunofluorescent staining and immunoassays for various proteins. Oligodendrocytes were also cultured on nanofiber plates to assess myelination and development. Cells were also treated with proteosome inhibitors to attempt rescue of HIKESHI protein and cellular function. Immunofluorescent staining indicates HSP70, and other proteins displayed altered nuclear localization in HIKESHI homozygous mutant cells. Bulk RNA sequencing was done during oligodendrocyte development and revealed downregulation in neurodevelopment genes and upregulation in heat shock genes. We also noted that homozygous mutant cells had fewer number of Olig2+ cells, aberrant myelination, and enlarged and less circular nuclei. Proteosome inhibitor treatment restored some HIKESHI protein and upon heat shock, cells displayed nuclear localization of HSP70 indicating that proteosome inhibitor treatment can restore some physiological function. Proteosome treatment over oligodendrocyte differentiation improved oligodendrocyte numbers and myelination in homozygous cells demonstrating therapeutic potential. We conclude that loss of HIKESHI results in heat shock pathway alterations and potential dysfunction of oligodendrocytes with proteosome inhibitors being a potential therapeutic. Due to the urgent clinical need, greater understanding would facilitate improved therapeutics to improve the quality and duration of patients with HAL.

25. Rumian, Nicole

CU Anschutz School of Medicine

Persistent restoration of LTP and behavior in 5xFAD mice by transient CaMKII inhibition

Long-term potentiation (LTP) is a form of synaptic plasticity thought to underly learning and memory. We have previously shown that CaMKII inhibition can prevent the impairment of LTP

mechanisms that is induced by Aβ1-3. Thus, in addition to the better-known role of CaMKII in acutely mediating physiological LTP4-7, CaMKII also mediates the pathological LTP impairments that are related Alzheimer's disease (AD) and this emerging dual role of CaMKII is subject of a recent review8. While mechanistically interesting, preventing Aβ-induced LTP impairments by CaMKII inhibition is not likely to be therapeutically relevant. However, we show here that CaMKII inhibition can also reverse the LTP impairment seen in the 5xFAD mouse model of AD: Transient incubation of hippocampal slices from 7-8-months-old 5xFAD mice with one of several mechanistically different CaMKII inhibitors (for 1.5 h) restored LTP when induced after washout of the drug (after 40 min to 1 h). By contrast, a similar inhibitor incubation of wild type slices did not enhance LTP, demonstrating specific reversal of disease-related LTP impairments rather than a non-specific augmentation of general LTP mechanisms. Maybe most importantly, with the CaMKII inhibitor tatCN19o, LTP restoration was observed even after in vivo injection; then, this LTP restoration lasted for at least one week. Thus, transient inhibition of CaMKII in 5xFAD mice in vivo can persistently restore the LTP impairments seen in this mouse model of AD. Similarly, initial experiments suggest restoration also of behaviors that are impaired in the 5xFAD mice, including in the open field and in the Y-maze. Notably, any direct negative effect of CaMKII inhibition with tatCN19o on learning are mild and very transient (fully reversed within 30 min), whereas the restorative effects of tatCN19o persist (for at least a week), thereby opening an unexpectedly extended time window of the therapeutic effect. The next steps for additional efficacy tests and drug development will be discussed.

26. Steinke, Kira

CU Anschutz School of Medicine

Encoding of position and odor concentration by neuronal activity in dorsal CA1 in mice engaged in odor plume navigation.

In nature, an animal's survival hinges on its ability to process and integrate sensory information, which is a critical skill for finding sustenance, avoiding threats, and selecting mates. An ethologically relevant behavior of particular interest is an animal's ability to detect, pursue, and ultimately reach target scents, termed "odor plume navigation", as this behavior relies on complex integration of present and past information of spatial and contextual experiences with intermittent olfactory information. Previous research suggests that the hippocampus, particularly the dorsal CA1 (dCA1) region, would be potentially a key player in odor plume navigation, because it is involved in spatial navigation and processes olfactory input from the lateral entorhinal cortex, a key location for odorant processing. In this work, we implanted GRIN lenses to image dCA1 pyramidal cell activity in Thy1-GCaMP6f mice using a miniscope as outlined in our previous publication (Simoes de Souza et al, JoVE doi:10.3791/67039, 2024), while recording mouse position in the arena. We found that following odor release, a mouse would consistently navigate towards one side wall, turn towards the spout, and increase velocity when it had chosen the side where the odorant was being released. We performed decoding of position and the logarithm of average odorant concentration using a binary decision tree machine learning algorithm. The algorithm was trained with z-normalized DF/F from all ROI activity in all but one trial and position and odorant concentration were predicted in the remaining trial (leave one out approach). For position decoding there were no differences in the goodness of fit between hit and miss trials

(goodness of fit was quantified as the correlation, R1, between predicted and actual values). In contrast, for prediction of odorant concentration R1 was higher for hits compared to misses. Interestingly, when ROIs were ranked in prediction importance the top 5% ROIs were largely non-overlapping between prediction of position vs. odorant concentration. The data indicates that dorsal CA1 participates in combined computation of position and odorant concentration in the odor plume navigation task.

27. Stewart, Amber

CU Anschutz School of Medicine

The role of subsynaptic nanoscale architecture in inhibitory synaptic plasticity

GABAergic inhibitory synapses mediate synaptic inhibition in the brain by regulating neuronal excitability, cell firing, dendritic integration, and synaptic plasticity – mechanisms which are critical for learning, memory, and cognition, and are disrupted in disease. Super-resolution imaging shows that GABA(A) receptors and the scaffold gephyrin form nanoscale subsynaptic domains or SSDs, which are thought to be important for synapse function by positioning receptors near sites of neurotransmitter release. We find that these SSDs are added to the synapse as it grows, suggesting a common mechanism for activity-dependent plasticity. Here we aim to investigate the fine details of synapse organization to understand how nanoscale subsynaptic architecture contributes to functional synaptic plasticity. This will begin to identify molecular interactions and structural mechanisms involved in the function and plasticity of inhibitory synapses and offer clues to how they are disrupted during disease.

28. Stine, Nathan

CU Anschutz School of Medicine

Role of Angiotensin II Type 1 Receptor in TRPC3-mediated constriction of pericytes from the arteriole-capillary transition zone.

Nathan C Stine1, Hannah R Ferris1, Felix Nguyen1, Fabrice Dabertrand1 1. University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States of America Background: Pericytes, a heterogenous population of mural cells found along the outside of the capillary lumen, have been shown to constrict in response to increased luminal pressure (Ferris, Sci Signal 2025). The bottlenecking of the arterial-capillary transition (ACT) zone positions the pericytes found in this region to be effective modulators of capillary resistance – a necessary mechanism for brain health. An increase in intraluminal pressure directly constricts ACT pericytes, reducing vessel diameter to regulate blood flow, demonstrating a myogenic-like response similar to that of arteriolar smooth muscle cells. However, the molecular pieces through which pericyte constriction occurs in the pressure signaling pathway is largely unknown. While the Transient Receptor Protein C3 (TRPC3) channel has been shown to depolarize pericyte membrane and cause subsequent constriction, upstream signaling is unknown. This study aims to test whether angiotensin II (Ang II) type 1 receptor (AT1R) activation recruits TRPC3 channel to cause pericyte constriction in the ACT zone. Hypothesis: ACT pericytes utilize the AT1R through Gq/11 signaling to engage TRPC3 channels, causing membrane depolarization and subsequent Ca2+ influx and constriction in response to pressure. Methods: Utilizing an ex vivo approach, we isolate and pressurized an intact arteriolar-capillary bed to examine a battery of stimuli on the vessels. Pressure myography was

employed to assess capillary diameter while confocal laser scanning microscopy measured requisite pericyte intracellular Ca2+ signaling from genetically encoded Ca2+ indicator mice. Increasing luminal pressure, genetic depletion of TRPC3 channels, as well as pharmacological regulation of AT1R and TRPC3 channels provided a basis for the pericyte contractile mechanism. Results: Our IHC results indicated that the capillaries had significant expression of the AT1R protein through positive staining. In ex vivo pressure myography, bath application of 100 nM Ang II induced a nearly 60% constriction of ACT pericyte. Genetic ablation of TRC3 channel virtually abolished this response. Consistent with the Ang II-induced constriction, we found that Ang II significantly increased intracellular calcium as well. In control conditions, increasing luminal pressure in 20 mmHg steps induced gradated myogenic tone responses. However, in presence of the AT1R inhibitor losartan (1 μM) as well as Gq/11 inhibitor FR900359 (1 μM), pericytes failed to develop pressure-induced constriction. This positions the Gq/11 -pathway in AT1R signaling for pressure dependent pericyte constriction. Conclusion: Our study demonstrates functional AT1R activates the Gq/11 – signaling pathway to activate TRPC3 dependent ACT pericyte contraction.

29. **Thorpe, Ryan**
CU Anschutz School of Medicine
Heterogeneous timescales of short-term synaptic plasticity mediate robust temporal representations
Neural circuits of the sensorimotor system produce temporally precise outputs in service of time-sensitive behavior. While it has been widely established that recurrent synaptic connectivity within the brain can produce learned spatiotemporal firing rate patterns with sub-second precision, artificial recurrent neural networks (RNNs) exhibit pronounced fragility to noise and aberrant input compared to their in vivo counterparts. It has been shown that RNN dynamics can be stabilized by explicitly constraining network connectivity to produce low-dimensional dynamics or exhibit innate, complex attractor states. Neural heterogeneity provides an additional boost in robustness by enriching a network's intrinsic timescales of activation. However, functional connectivity within the brain rarely remains constant over the sub-second timescale due to short-term synaptic plasticity (STP). Time-variant connectivity presents a unique confound for neural encodings that rely on recurrent activity, as it ostensibly makes the downstream response of anticipated neuron-to-neuron interactions even more sensitive to minuscule perturbations. As such, the stability and robustness of learned network responses imposed by the intrinsic timescales of dynamic synaptic connections remain poorly understood. Here, we explore the possibility that biologically inspired distributions of heterogeneous STP across the units of an RNN not only stabilize learned temporal responses, but provide a repertoire of intrinsic timescales for encoding temporal information ideally suited for sensorimotor tasks.

30. **Wolfe, Sarah**
CU Anschutz School of Medicine
Excitation-transcription coupling mediates sustained GABAergic synaptic plasticity
Late stages of inhibitory long-term potentiation (iLTP) result in de novo synthesis of inhibitory synaptic proteins that can sustain long-term changes in synaptic strength. The microRNAs, miR376c and miR153, repress translation of GABAaRs and gephyrin under basal conditions, but following iLTP miRNA transcription is repressed, enabling increased translation of GABAaRs and

gephyrin. This repression of miRNA expression is regulated by the transcription factor NFAT, providing a novel pathway to reduce miRNA expression during inhibitory synaptic plasticity. Here, I will further characterize NFAT translocation following iLTP and if NFAT is required for inhibitory synaptic potentiation.

31. **Yee, Andrew**
    CU Anschutz School of Medicine
    Precise spatiotemporal encoding of striatal dopamine signals
    Striatal dopamine (DA) signaling is traditionally thought to be mediated by a spatially broad mode of transmission, whereby low concentrations of DA non-selectively activate extra-synaptic receptors. However, this mode of signaling is not consistent with behaviors mediated by DA which require high spatiotemporal precision, as well as increasing evidence indicating that striatal DA transmission is more tightly organized than previously appreciated. Using two-photon imaging and whole-cell electrophysiology, we have directly defined the spatiotemporal properties of DA transmission onto indirect pathway spiny projection neurons in striatal brain slices. Sparse activation of DA release sites evoked highly localized DA signals which produced spatially-discrete, D2 receptor-mediated responses via Gβγ-mediated signaling. Membrane-delimited Gβγ signaling required higher concentrations of DA compared to downstream second messenger signaling (i.e. Δ[cAMP]). Spatially-restricted DA signals were diversely encoded at dendrites of striatal neurons, indicating that subcellular localization is critical for signal encoding. We propose that membrane-delimited Gβγ signaling occurs in parallel to classical second messenger pathways, providing a mechanism for precise spatiotemporal encoding of behaviors by striatal DA transmission.

32. **Garcia, Nicholas**
    CU Anschutz School of Medicine
    A Computational Framework for Characterizing Neural Codes
    How neurons encode information is the key central question of neuroscience. While the discovery of single neuron spatiotemporal feature selectivity was an important milestone in our understanding of the visual system, it was quickly recognized that visual neurons are modulated by a growing list of contexts that include visual, cross-sensory, motor, arousal state, and behavioral task-related. Such “mixed-selective” visual neurons exhibit tuning for multiple independent variables and have highly complex feature selectivity. These findings have emphasized the importance of population level features to neural computation. Central to this question is the neural code the brain uses to represent visual information. We have developed a theoretical and analytical framework for characterizing the visual code used by both artificial and biological neural networks and show our validation of the approach on a representative model. Our approach consists of shallow multi-task learning convolutional neural networks trained to reproduce the spatiotemporal statistics of random dot kinetograms and drifting gratings. This architecture allows us to ask about how resources are allocated within shared layers, which we quantify by characterizing single unit and population metrics such as selectivity, linearity, dimensionality, and decoding performance. We plan to apply this framework to orient the mammalian visual cortical code within a landscape of artificial vision models. By training families of computational models with varying architecture, we can characterize how constraints shape the neural code used to solve simple visual discrimination tasks.

NTC Core posters

33.The NTC Advanced Light Microscopy Core

The Advanced Light Microscopy Core (ALMC) offers a broad range of microscope systems and techniques. Here we give an overview of the capabilities which range from basic brightfield and epi-fluorescence imaging to high content and super-resolution imaging.

34.The NTC Animal Behavior/In-Vivo Neurophysiology Core

The **Animal Behavior and In Vivo Neurophysiology Core** provides CU Anschutz investigators with expert support and advanced facilities for studying behavior and brain function in animal models. Researchers gain access to specialized equipment, consultation, and training to design, execute, and analyze experiments efficiently. These resources help ensure high-quality, reproducible data that accelerate discovery in neuroscience and related fields.

NTC Neural Engineering Core

35. IDEA Core

The Innovation and Design for Experimentation and Analysis Core specializes in the design and implementation of large-scale, integrated systems for acquiring and analyzing neuroscience data. If you have an idea for an experiment, but the ideal apparatus is not available commercially, then the IDEA core is here to help.

36. **The NTC Machine Shop: Partnering with Neuroscience Researchers**

The NTC Machine Shop supports Neuroscience Researchers by turning ideas into practical tools for discovery. We provide custom fabrication and prototyping to meet the unique needs of each project—whether that means designing specialized fixtures, creating rapid prototypes, or adapting equipment for new experiments. Our goal is to offer accessible, cost-effective solutions that help you focus on your research while we handle the technical challenges.

37. ONE Core

The mission of the Optogenetics and Neural Engineering (ONE) Core is to provide custom engineering support to the neuroscience community at the University of Colorado Anschutz Medical Campus.

38. Software and Data Management Support for Neural Research

I recently joined the NTC as part of the Neural Engineering core as a resource for the creation/update of programming and software development tools. My professional background is primarily in Python development, with hands-on experience in cloud computing environments, version control systems such as Git, and the integration of artificial intelligence tools and frameworks. I have worked on a variety of technical projects, ranging from backend development to deploying scalable cloud-based applications. I hold a bachelor's degree in neuroscience from the University of Michigan and am looking forward to applying my interdisciplinary skills to help design and build any programmatic tools and infrastructure that you may need.