2015 SCSB FALL POSTER SESSION ABSTRACTS

Functional evaluation of bipolar- and schizophrenia-associated variation in the voltage-gated calcium channel CaV3.3

A. ALLEN, H. YAN, Y.-L. ZHANG, J. Q. PAN
Presented by: Andrew Allen

Functional characterization of genetic variation is essential for utilizing emerging genomic data to understand the genetic risk underlying human diseases and to aid in the development of targeted therapeutics. Currently, computational approaches are unable to reliably predict the functional impact of genetic variation, necessitating the development of high-throughput, biologically-relevant assays to rapidly evaluate the functional impact of many genetic variants simultaneously. This is especially difficult to achieve for genes implicated in neuropsychiatric disease, as relevant and reliable in vitro or ex vivo systems can be difficult to establish, and the phenotypic assessment of genes required for neuronal function is not always amenable to high-throughput methodologies. The T-type low-voltage-activated calcium channel CaV3.3 has been implicated in schizophrenia risk by exome sequencing in trios and in genome-wide association studies. In order to understand the impact of sequence variation on the function of CaV3.3, we identified a series of coding variants found in bipolar patients, schizophrenic patients, and control subjects identified by exome sequencing in a Swedish cohort. Subsequently, we established a heterologous expression system of isogenic cell lines carrying single-copy inducible cDNA variants of CaV3.3 and evaluated their functional impact on channel function by high-throughput electrophysiology, calcium imaging, and biochemistry. Our results demonstrate that several patient-associated variants impair CaV3.3 channel function by reducing overall protein abundance and/or membrane surface expression. Furthermore, we establish a framework for the high-throughput evaluation and interpretation of genetic variation in ion channels that can be implemented for the rapid functional evaluation of mutations associated with neuropsychiatric disease.

Expansion Sequencing (ExSEQ): Comprehensive In Situ Transcriptome Characterization Throughout Intact Brain Circuits

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Presented by: Shahar Alon

Enabling the mapping of the cell types of the brain, as well as the systematic analysis of cell types in complex behavioral and disease states, would benefit greatly from a technology for the comprehensive analysis of gene expression patterns in neurons throughout a neural circuit. Ideally we could perform this analysis in intact brain circuits, so that these transcriptional profiles could be combined with morphological and circuit topology information, resulting in unified pictures of the cell types and cell states of the brain. Current tools do not permit this: optical methods maintain the spatial location of molecules, but the number of molecules that can be studied simultaneously is limited. On the other hand transcriptomic approaches allow the multiplexed measurement of potentially all the RNA molecules, but spatial information is lost in the process. Here we devise a new method for in situ sequencing of nucleic acids throughout all the neurons of an intact brain circuit, by creating new forms of expansion microscopy (ExM), a technology we previously developed that physically magnifies brain

tissues while preserving nanoscale isotropy (Science 347:543-548), as well as fluorescent in situ sequencing (FISSEQ; Science 343:1360-1363). Using this new technology, which we call expansion sequencing (ExSEQ), users can expand brain circuits, then sequence the RNAs within the expanded tissue, resolving transcripts throughout entire neurons and neural circuits, enabling systematic cell type and cell state classification in health and disease.

Integration and Segregation of Default Mode Network Functional Connectivity in High-functioning Autism Spectrum Disorder

S. A. ANTERAPER, S. WHITFIELD-GABRIELI, J. BIEDERMAN, J. GABRIELI, K. CONROY, R. KILCULLEN, J. NISSEN, G. JOSHI

Presented by: Sheeba Arnold Anteraper

The default mode network (DMN) is noted as an integrated system that supports self-monitoring and social, emotional, interpersonal, and introspective processes[1]. Although the DMN is the most extensively studied resting-state network in autism spectrum disorder (ASD) with most consistent finding of reduced resting-state functional connectivity (RsFc) within the DMN with weaker coherence of RsFc between the posterior and anterior subsystems, yet simultaneous examination of both, positive and negative correlations of intrinsic functional connectivity remains unexplored[2, 3]. We hypothesize that individuals with ASDs will exhibit atypical profile of both, integrative (positive) and segregative (negative) RsFc correlations of DMN. Using seed-based functional connectivity MRI, we assessed the RsFc profile of DMN in young adults with ASD (N=15) in comparison with healthy controls (N=16) with the aim to examine the integration and segregation strength of the intrinsic functional organization of DMN. Results reveal that the intrinsic functional organization of DMN is significantly altered in ASD as manifested by weaker integration and segregation of the network. Future studies with larger sample sizes are warranted.

References:

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Gtf2i-deletion affects social behavior, neuronal morphology and the transcriptome in a novel mouse model for Williams syndrome

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Presented by: Boaz Barak

Williams syndrome (WS) is a neurodevelopmental disorder caused by a chromosomal microdeletion of about 26 genes from the WS chromosomal region (WSCR) in 7q11.23. In contrast to ASD, WS patients demonstrate enhanced sociability with relatively preserved language skills, and impaired cognition. Recent studies of WS patients with partial deletions suggest that specifically the deletion of the general transcription factor II-i (Gtf2i) may play a key role in social behavior.

To study the role of Gtf2i in social behavior, we generated a Gtf2i conditional knockout mouse. By so, we were able to induce germline deletion of the Gtf2i gene and study the behavioral and biochemical consequences of it. We found that Gtf2i-haploinsufficiency results in developmental impairments and substantial abnormalities in social behavior, with hypersocial behavior and lack of social habituation, as compared to WT littermates. Gtf2i full knockout from neurons resulted in developmental arrest of the dendritic tree of neurons but not their dendritic spines. This developmental abnormality is supported by RNAseq results, showing substantial abnormalities of the transcriptome in development-related processes. Full knockout of Gtf2i from principal neurons of the cortex and hippocampus resulted in significantly lower brain weight together with increased neuronal density in the mouse cortex, and significantly higher levels of social behavior, anxiety and impaired novel object recognition.
Overall, our data suggest that Gtf2i-deletion specifically is responsible for the behavioral and anatomical phenotypes in full WSCR-deletion WS patients.

Use of CRISPRs to Remove of a Spontaneous Mouse Mutation Causing Abnormal Anxiety- /Autism-like Behaviors
F. J. BUSTOS, S. PANDIAN, F. ZHANG, M. CONSTANTINE-PATON
Presented by: Fernando J. Bustos

The spontaneous mutant mouse *Flailer* has seizures that end at ~P27 and beginning in young adulthood shows high anxiety- and Autism-like behaviors caused by a spontaneous recombination event that places a brain specific promoter (*gnb5*), in frame with the exons for the cargo binding domain of the f-actin motor protein Myosin Va. Myosin Va normally transports the scaffolds for glutamate receptors to spine synapses. Whole body mutations of *myo5a* cause early death in rodents (Mercer et al., 1991) and in humans *myo5a* mutations cause neurological dysfunction, mental retardation, hypomelanation and death in infancy or childhood (Griscelli et al., 1978 and Elejalde et al., 1979). The abnormal *Flailer* behavior is only present when this extra gene called *flr* is in a 1:1 ratio with WT *myo5a* it functions as a dominant negative, resulting in animals with defective synapse elimination and the abnormal behaviors (Jones et al., 2000, Yoshii et al., 2013). Removing the *flr* gene from specific brain regions would allow analyses of the mechanisms and pathways involved in the abnormal behaviors of *Flailer* mice.
Recently the use of CRISPRs has emerged as a technology that is able to edit the genome and eliminate specifically targeted sequences. Using double nicking CRISPRs we were able to specifically target the *flr* genomic sequence removing a fragment of the *flr* gene without altering the endogenous DNA sequences of either *myo5a* or *gnb5*. This deletion produced a significant decrease in both mRNA and protein levels in *Flailer* neurons without altering levels of WT Gnb5 or MyoVa. Immunofluorescence experiments using antibodies against PSD95 and Synaptophysin demonstrate that clustering of these synaptic proteins is recovered in neurons expressing the CRISPRs designed to delete the *flr* gene.
Viral introduction of these CRISPRs into specific brain regions will be used to unambiguously determine the circuits involved in the abnormal behaviors presented by *Flailer* mice.

Next-generation expansion microscopy: 20-nm resolution imaging via physical specimen magnification
J.-B. CHANG, F. CHEN, E. JUNG, H. BABCOCK, A. WASSIE, P. TILLBERG, X. ZHUANG, E. BOYDEN
Presented by: Jae-Byum Chang

The identification and localization of proteins and other biomolecules, throughout entire brain circuits, with nanoscale precision would enable many fundamental insights into the mechanisms underlying the operation of normal and pathological neural networks. We recently discovered that we could physically

magnify specimens by embedding them in a dense swellable polymer, anchoring key biomolecules to the polymer mesh, and adding water to swell the polymer, a process we call 'expansion microscopy' (ExM; Science 347(6221):543-548). Despite the high isotropy of the expansion process, the initial polymer recipe enabled just 4-4.5x expansion, or roughly 60-70 nm spatial resolution. Ideally it would be possible to improve the expansion chemistry so as to enable, ultimately, the imaging of membrane boundaries, as well as protein complexes. Here, we report on a next-generation ExM chemistry that can achieve ~15-20x physical magnification of mouse brain tissues, or 20-nm lateral resolution on conventional optical microscopes. As with the first version of ExM, next-generation ExM-processed samples are optically clear. Thus, next-generation ExM may be useful for imaging nanoscale neuronal structures such as synaptic clefts or synaptic vesicles over entire neural circuits in intact mammalian tissues. Brain circuit mapping using next-generation ExM may open up a variety of insights into the underpinnings of behavior, cognition, and disease. We continue to refine the chemistry and to explore how affinity tags can be adapted to work in this new expanded environment.

Oxytocin Mediates Entrainment of Sensory Stimuli to Social Cues of Opposing Valence
H. K. CHOE*, M. D. REED*, N. BENAVIDEZ, D. MONTGOMERY, N. SOARES, Y. S. YIM, G. CHOI
Presented by: Han Kyoung Choe, Michael D. Reed

Meaningful social interactions modify behavioral responses to sensory stimuli. The neural mechanisms underlying the entrainment of neutral sensory stimuli to salient social cues to produce social learning remain unknown. We used odor-driven behavioral paradigms to ask if oxytocin, a neuropeptide implicated in various social behaviors, plays a crucial role in the formation of learned associations between odor and socially significant cues. Through genetic, optogenetic, and pharmacological manipulations, we show that oxytocin receptor signaling is crucial for entrainment of odor to social cues but is dispensable for entrainment to nonsocial cues. Furthermore, we demonstrate that oxytocin directly impacts the piriform, the olfactory sensory cortex, to mediate social learning. Lastly, we provide evidence that oxytocin plays a role in both appetitive and aversive social learning. These results suggest that oxytocin conveys saliency of social stimuli to sensory representations in the piriform cortex during odor-driven social learning.

Integrating roles of the cytoskeletal regulator Farp1 in synapse and dendrite development
A. COLEMAN, T. BIEDERER
Presented by: Andrew Coleman

Genetic studies have linked mutations in genes regulating synapse development and the actin cytoskeleton to autism spectrum disorders and intellectual disability. A recent study identified an autism-linked, *de novo* frameshift mutation in the actin cytoskeleton regulatory protein Farp1. We have previously demonstrated that Farp1 regulates excitatory synapse development and activity-dependent dendrite development in maturing hippocampal neurons. Preliminary results suggest that the autism-linked mutation in Farp1 affects protein stability. Loss of function analysis will characterize the contribution of Farp1 to *in vivo* dendrite development and cognitive development. Functions of Farp1 in dendrite and synapse development will be ascribed to domains and signaling partner interactions, and disease-relevant roles of Farp1 downstream of its binding partners SynCAM 1 and Plexin A1 will be tested. These studies aim to determine how aberrant postsynaptic signaling contributes to the pathophysiology of autism disorders.

Probing brain functional connectivity in rat models of autism
M. DESAI, S. BRICAULT, A. FIALLOS, A. JASANOFF
Presented by: Mitul Desai

Autism spectrum disorders (ASDs) appear to be marked by altered correlations in brain-wide activity patterns observable by functional magnetic resonance imaging (fMRI) and electroencephalography. Analysis of these "functional connectivity" (FC) differences could contribute to understanding the pathology of autism, and might also yield biomarkers useful for clinical diagnosis and treatment monitoring. At the same time, FC differences observed in humans are difficult to interpret in mechanistic terms, and their relationship to specific ASD-related genetic lesions or systems-level physiological perturbations are difficult to discern. It would therefore be of great value to establish FC measures in rodent models where complementary behavioral and physiological techniques are also applicable. This proposal seeks to apply task-independent resting state FC analysis to characterize brain networks in awake rats in the presence or absence of genetic and chemical perturbations predicted to induce or probe ASD-related phenotypes. This work will test the hypothesis that defined interventions in experimental animals can recapitulate and explain FC results observed previously in humans, and will establish a model system suitable for systematic experimental investigations of whole-brain neural activity phenotypes in autism.

Combinatorial image-based screening with CRISPR/Cas
D. FELDMAN, F. ZHANG, P. BLAINEY
Presented by: David Feldman

Functional genomics analyzes the effect of genetic state on cellular function. Large-scale genetic perturbation assays use parallel DNA synthesis, cloning and lentiviral delivery to study up to 100,000 perturbations in one high-throughput assay. Recent developments in CRISPR/Cas9 allow construction of libraries to test perturbations in the native genomic context. However, genome-wide screens are limited to selection-based readouts (e.g., cell viability or expression of a fluorescent marker), precluding fluorescence microscopy-based readout of protein levels, gene expression, and cellular sub-structure and morphology. Here we are developing a purely optical method to uniquely identify libraries of up to 100,000 different viral vectors via expression of an RNA barcode. We aim to apply this method to dissect all pairwise interactions among the top 50 ASD-related genes in a differentiated neuronal model.

Expansion Microscopy in Zebrafish
L. FREIFELD*, O. RANDLETT*, I. ODSTRCIL*, D. MATRIN-ALARCON*, J. GAGNON*, A. SCHIER, F. ENGERT, E. BOYDEN (*these authors contributed equally to this work)
Presented by: Limor Freifeld

We recently discovered that it was possible to physically magnify samples by embedding them in a dense swellable polymer, performing a series of chemical treatments, and then swelling the polymer (and thus the sample), a process we call expansion microscopy (ExM; Science 347(6221):543-548). Thus, one can image large 3-D samples, e.g. entire brain circuits, with nanoscale precision using ordinary diffraction-limited microscopes. Expansion microscopy enables imaging of fine details of neural projections and synaptic protein distributions, while preserving the contextual information of entire cell and circuit structures. We here report on the application of expansion microscopy to the study of the larval zebrafish, a key organism in neuroscience. The larval zebrafish is a transparent vertebrate whose

brain spans only ~500µm X 500µm X 1000µm in volume. We expand entire brains of 5-7 dpf larval zebrafish and examine the detailed structure of different neural populations at multiple spatial resolutions throughout the entire brain. We follow neural projections across brain regions and describe their fine inter-region distributions and synaptic contacts at a resolution exceeding the diffraction limit, across volumes that span the entire central nervous system. As this vertebrate model lends itself to whole-brain functional imaging (Ahrens et al., 2012, Ahrens et al., 2013, Prevedel et al., 2014), applying expansion microscopy to larval zebrafish may enable rapid analysis of connectivity information. This, in the context of interpreting neural activity, may facilitate better mechanistic understanding of neural information processing in complete neural circuits. We also demonstrate expansion microscopy of zebrafish embryos, enabling high-resolution imaging of different developmental stages.

Multiplexed neuronal synapse imaging using PAINT
S.-M. GUO, R. VENEZIANO, S. GORDONOV, D. PARK, T. KULESA, P. BLAINEY, E. BOYDEN, M. BATHE
Presented by: Syuan-Ming Guo

Neuronal synapses form critical junctions of communication in neuronal networks, mediating neuronal signal transmission and circuit function. Synapses consist of thousands of proteins organized on the sub-micron scale, and their dysregulation via genetic aberrations including copy number variations and site-specific mutations is associated with a large number of neurological and psychiatric diseases. Understanding how these genetic aberrations affect the localization and structural organization of synapse proteins at the single synapse level is crucial for understanding neuronal function and related pathogenesis. Super-resolution fluorescence imaging is a powerful approach to resolving nanometer-scale organization of synapse molecules. However, conventional super-resolution imaging is limited to simultaneous interrogation of only 2-4 proteins in a single synapse. As an alternative, here we apply DNA-PAINT (Points Accumulation for Imaging in Nanoscale Topography) that enables highly multiplexed super-resolution imaging of synaptic proteins. We employs transiently binding DNA probes to molecular targets conjugated with complementary DNA sequences in order to generate target blinking while simultaneously allowing probe wash-out or exchange, thereby in principle enabling sequential imaging of arbitrary numbers of molecular targets using a single dye and laser source. Co-localization analysis of the antibody staining patterns with the synaptic marker synapsin-1 shows no significant staining pattern change for most synaptic antibodies, suggesting the affinities of the antibodies are not altered by DNA conjugation. We employ DNA-PAINT to resolve the localization and organization of ~10 targets simultaneously including synaptic proteins and cytoskeletal markers. Distributions of synaptic proteins within individual synapses are consistent with previous electron microscopy and three-color super-resolution imaging studies.

Shank modulates postsynaptic Wnt signaling to regulate synaptic morphology and function
K. P. HARRIS, R. W. CHO, Y. AKBERGENOVA, M. S. BAAS-THOMAS, J. T. LITTLETON
Presented by: Kathryn P. Harris

Prosap/Shank scaffolding proteins regulate the formation, organization, and plasticity of excitatory synapses. Mutations in SHANK family genes are implicated in autism spectrum disorder (ASD) and other neuropsychiatric conditions. However, the molecular mechanisms underlying Shank function are not fully understood, and no study to date has examined the consequences of complete loss of all Shank proteins in vivo. Here we characterize the single Drosophila Prosap/Shank family homolog. Shank is enriched at the postsynaptic membrane of glutamatergic neuromuscular junctions and functions in a

dose-dependent manner to control multiple parameters of synapse biology. Loss or overexpression of Shank results in defects in synaptic maturation, transmission, and structural plasticity. We demonstrate that Shank regulates a non-canonical Wnt signaling pathway in the postsynaptic cell by modulating internalization of the Wnt receptor Fz2. This study identifies Shank as a key synaptic component of Wnt signaling, defining a novel mechanism for how Shank contributes to synaptic maturation during neuronal development.

Mouse cingulate cortex coordinates visuomotor function
R. HUDA, G. PHO, I. WICKERSHAM, M. SUR
Presented by: Rafiq Huda

Neural dynamics in sensory cortices are shaped by bottom-up inputs relaying the physical features of sensory stimuli and by top-down projections that modulate their encoding. The anterior cingulate division of the prefrontal cortex is known to provide top-down input to the visual cortex. Here, we use multiple approaches to delineate the functional role of visual inputs to the anterior cingulate cortex (ACC) and of the feedback from ACC to V1. Using rabies virus-mediated anatomical tracing to identify sources of inputs to the ACC, we found that V1 as well as other cortical and subcortical brain regions project to the ACC. Using rabies viruses that express the genetically encodable calcium indicator GCaMP6f and two-photon microscopy, we characterized the functional properties of ACC-projecting visual cortex neurons in passively viewing, awake head-fixed mice. We found that many of these neurons are tuned to the orientation and direction of drifting gratings. Next, we expressed GCaMP6s in the ACC and imaged the calcium activity of ACC axons found in layer 1 of V1. A subset of ACC axons were visually driven and displayed sharply tuned responses to the orientation and direction of drifting gratings. To assess the contribution of V1 to this property, we used a chemogenetic approach. We expressed the inhibitory hM4Di DREADD (designer receptors exclusively activated by designer drugs) in V1, GCaMP6s in the ACC, and monitored the calcium responses of ACC axons to oriented drifting gratings before and after systemic application of the DREADD agonist clozapine-N-oxide (CNO). While CNO application in control animals had no effect on the visual responses of ACC axons, it reduced responses in DREADD expressing animals. Together, these findings show that a projection from the visual cortex contributes to the visual responsiveness of the ACC. Since the ACC has been proposed to play a crucial role in cognition, and in particular reward processing, we propose that the ACC processes visual information in the context of its behavioral significance and relays a saliency signal back to the visual cortex to modulate the encoding of relevant visual stimuli.

Human and Mouse Models of Rett Syndrome exhibit altered prenatal cortical development due to alterations in neurogenesis
P. IP, N. MELLIOS, D. FELDMAN, S. D. SHERIDAN, S. KWOK, B. ROSEN, B. CRAWFORD, Y. LI, R. JAENISCH, S. J. HAGGARTY, M. SUR
Presented by: Jacque Ip

Rett Syndrome (RTT) is a neurodevelopmental disorder that, in the vast majority of cases, arises from mutations in the X-linked gene MECP2. MeCP2 is an epigenetic modulator of gene expression that has recently been shown to interact with miRNA machinery. In addition, MeCP2 itself has been implicated in several neurodevelopmental disorders. Multiple lines of evidence point to the importance of miRNA-mediated pathways downstream of MeCP2 in different stages of brain development and plasticity. We hypothesized that the pleiotropic effects of MeCP2 in prenatal development are mediated via a set of

early regulated miRNAs. Towards that end, we used induced pluripotent stem cell (iPSC) RTT lines generated from patients, virally-mediated knockdown of MeCP2 in human embryonic stem cells (ESCs), TALEN-derived isogenic ESC RTT lines, and an Mecp2 mutant mouse model as complementary approaches to identify novel MeCP2-regulated miRNAs and examine their respective influence on neurogenesis and neuronal differentiation.. Via BrdU pulse labelling, we found that the proliferation rate of patient-derived and MeCP2-deficient neuronal progenitor cells was significantly altered relative to control cells; this was accompanied by reductions in the expression of early neuronal markers and immature dendritic morphology. Our findings to date implicate aberrant regulation of prenatal neurogenesis as a result of MeCP2 deficiency. Taken together, our data support a novel miRNA-mediated pathway downstream of MeCP2 capable of influencing neurogenesis via interactions with central molecular hubs linked to autism spectrum disorders. Ongoing experiments are focused on elucidating the mechanisms of disease-related impairments in neurogenesis in both mouse and human organoid models of RTT, and translating these findings to scalable assays for novel therapeutic discovery.

Can Blindness Cause Autism?
W. JAMAL, A. CARDINAUX, L. DENNA, S. GUTNIK, M. KJELGAARD, P. SINHA
Presented by: Wasifa Jamal, Annie Cardinaux

Numerous empirical and theoretical perspectives point to a possible link between blindness and ASD. The existence and mechanisms responsible for such a link are of interest not only from the applied perspective of early risk assessment and care, but also for understanding basic issues related to the pathogenesis of autism. A recent study by Jure and colleagues (2015) estimated a 48% prevalence of autism in blind children, and ascribed a causal role to blindness. There are, however, alternative explanations for higher rates of ASD diagnosis in the blind, (Baron-Cohen, 2002; Bambring, 2011; Sinha and Leonova, 2011) and some accounts also indicate that “autistic" traits identified in the blind may be etiologically distinct from autism per se. In this poster, we shall review the empirical evidence thus far, the inferences that the data can support and possible approaches for rigorously investigating the relationship between blindness and autism.

The Nature of Pragmatic Impairment in Autism Spectrum Disorders
O. JOURAVLEV, A. PAUNOV, I. BLANK, Z. MINEROFF, E. FEDORENKO
Presented by: Olessia Jouravlev, Alex Paunov

The ability to exploit speaker intent and background linguistic and social knowledge in order to go beyond the literal meaning of the sentence is called “pragmatics”, and it is at the core of the communication deficit in autism spectrum disorders (ASD). In our research, we use a synergistic combination of functional neuroimaging (fMRI) and behavioral approaches to characterize the nature of the pragmatic impairment in ASD. Preliminary results of four ongoing studies will be discussed. The aim of Study 1 was to examine psychometric properties of three most commonly used tests of social abilities: ASQ, Reading the Mind in the Eyes, and Reading the Mind in the Movies tasks. Study 2 entailed examination of the performance of non-ASD individuals in a wide range of tasks tapping into pragmatic processing (N = 20). The goals of Study 2 were (a) to reveal the latent structure of pragmatics and (b) to assess the contributions of some cognitive and social skills (e.g., intelligence, prevalence of autistic features, preferred learning styles etc.) to efficiency of pragmatic processing. In Study 3, we examined correlations in the patterns of brain activity across the Language and Theory of Mind systems, systems

that should work in synchrony during pragmatic processing, during comprehension of short stories. In Study 4, the correlations in the BOLD signal fluctuations across brain regions of the Language, Theory of Mind, and Cognitive Control systems during comprehension of a set of naturalistic stimuli, involving a high vs. low degree of pragmatic reasoning, were examined. At this point, all studies were conducted with non-ASD individuals. Our next step is to examine behavioral performance and fMRI patterns of brain activity of ASD individuals completing Studies 2 – 4.

Immunostaining and morphological analysis of human iPSC-derived cortical neurons
K. MURUGADOSS, L. HOGSTROM, F. RAPINO, S. LIPNICK, L. RUBIN. M. BATHE
Presented by: Karthik Murugadoss

Methods to relate molecular measurements of synaptic composition to neuronal morphology will be crucial for improving functional and developmental models of the brain. Synaptic proteins regulate a range of activities central to neurotransmission such as ion homeostasis, trans-synaptic cell-adhesion and vesicle-mediated neurotransmitter release. Mutations in presynaptic and postsynaptic proteins have been linked to neuropsychiatric disorders such as schizophrenia and autism spectrum disorder. Induced pluripotent stem cells (iPSCs) can be derived directly from patient-specific somatic tissue and represent an exciting tool to explore the influence of genetic variation on neuronal development. Here, we focus on in situ phenotypic profiling and associated analyses of iPSC cortical neurons. Immunofluorescence is used to evaluate targets that could be used for multiplexing strategies such as PAINT. Colocalization of synaptic as well as structural proteins is quantified through the Pearson correlation coefficient. Punctae measurements of synaptic markers provides additional insight on synaptic protein abundance.

Development of a New Behavioral Assay of Visual Memory in Mice
R. PARKMAN, R. KOMOROWSKI, M.F. BEAR
Presented by: Rachael Parkman

The ability to assess high-level cognition and memory in mice and then determine the network of brain regions involved is a critical first step towards the ultimate goal of using mouse models to better understand the neurobiology of cognitive disorders. Previous work assessing visual memory in the mouse has provided evidence that the primary visual cortex of the mouse is one key region where long-term visual memories are stored and that manipulations of this region impair the ability of the animals to selectively habituate to a visual stimulus. Despite its success, this method is limited in that the mouse is head-fixed and not able to naturally explore the stimulus and that it requires a long training period. The primary goal of this investigation is to optimize the newly proposed behavioral assay, Freely Moving Orientation Specific Habituation or FreeMOSH. To do so, we condensed the training protocol, improved our measurements of relevant behaviors, and assessed the selectivity of this visual memory. Furthermore, we find that this task depends upon NMDA-Receptors within primary visual cortex, suggesting that plasticity within this region is critical for formation of this visual memory.

Flexible task-dependent transformations in mouse parietal cortex during sensorimotor decisions
G. PHO, M. GOARD, M. SUR
Presented by: Gerald Pho

The posterior parietal cortex (PPC) has been implicated in perceptual decisions, but its specific role at the interface between sensation and action remains unresolved. Here, we trained mice on a visual decision task with distinct stimulus and motor epochs, and optically silenced or recorded activity during these epochs. Inactivation experiments revealed that both primary visual cortex (V1) and PPC were necessary during the stimulus period, but not for execution of the motor response. Using two-photon calcium imaging during both engaged and passive viewing trials, we found that, unlike V1, most neurons in PPC responded exclusively during engaged task performance trials with a strong bias towards target stimuli. A subset of PPC neurons, however, had significant responses during both engaged and passive conditions that depended on stimulus contrast. Engagement-modulated neurons and contrast-modulated neurons were mostly distinct subpopulations that were spatially intermingled within PPC. Lastly, we re-trained mice with a reversed reward contingency, and imaged the same neurons before and after the reversal. We found that most PPC neurons exhibited a dramatic shift in selectivity towards the new target stimulus, but the subset of neurons with weak behavioral modulation maintained their stimulus selectivity. Together these results point to a functional heterogeneity in mouse PPC that enables the flexible transformation of sensory inputs into motor commands.

Dissecting the inhibitory mechanisms of reliable coding in mouse V1
R. V. RIKHYE, M. SUR
Presented by: Rajeev V. Rikhye

Neurons in the primary visual cortex (V1) respond to full-field natural scenes with spike trains that are highly reliable between trials. While it has been argued that local inhibitory interneurons are responsible for modulating reliable coding, no study has yet systematically detailed the role of different interneuron subtypes. Our goal was to show how Parvalbumin (PV), Somatostatin (SST) and Vasoactive Intestinal Peptide (VIP) expressing interneurons modulate reliable coding in mouse V1. Specifically, we aimed to: (1) show how subnetworks of these interneurons process natural scenes and (2) determine how they contribute to reliable coding.

To address these questions, we performed *in vivo* two-photon calcium imaging in awake, head-fixed mice by conditionally expressing GCaMP6f in PV, SST or VIP neurons. This allowed us to minimize the effect of contamination from nearby excitatory neurons and permitted us to study population coding within these interneuron subnetworks. SST neurons also preferred lower spatial frequencies than PV neurons, consistent with their role in integrating information from a larger visual area. Not surprisingly, VIP neurons responded poorly to gratings.

PV neurons responded strongly, but unreliably, to full-field natural scenes. In contrast, SST neurons were more selective and were highly reliable between trials. SST cell reliability was comparable to excitatory neurons. This suggests that SST neurons are selectively driven by specific features in natural scenes and, consequently, provide reliable dendritic inhibition on their target cells. We also found that VIP neurons responded more strongly to natural scenes than gratings, suggesting that these interneurons are driven more by “salient” stimuli.

Next, we investigated how these interneurons modulated pyramidal cell reliability. To do so, we conditionally expressed ChR2 in both PV or SST neurons and GCaMP6f in pyramidal neurons. We reasoned that reliability arose due precisely timed excitatory (E) and inhibitory (I) synaptic currents. Thus, we used a stimulation protocol to decorrelate these E- and I-currents in pyramidal cells.

Specifically, we pulsed a blue LED for 100ms at random times during a natural movie. This allowed us to activate PV/SST neurons during periods when pyramidal cells were most reliable. We discovered that activating SST neurons during epochs of reliability increased reliability. In contrast, stimulating PV neurons reduced reliability.

Taken together, our work demonstrates that SST neurons play an important role in shaping the reliability of pyramidal cell responses to natural scenes in mouse visual cortex.

High-Density Electrode Arrays for Neural Recording

J. SCHOLVIN, J.P. KINNEY, J.G. BERNSTEIN, C. MOORE-KOCHLACS, N. KOPELL, C.G. FONSTAD, E.S. BOYDEN

Presented by: Joerg Scholvin

Our research focuses on strategies for designing and fabricating three-dimensional microelectrode arrays, to be used for extracellular neural recording. Our designs have customizable electrode locations, targetable to specific neural substrates, and distributed in a volume throughout a neural network in the mammalian brain. We accomplish this by utilizing MEMS microfabrication techniques to create a number of planar structures that are mechanically and electrically assembled into a three-dimensional array (Figure 1). The resulting array consists of a large number of thin needles, and each needle contains many recording sites along its length. To pick up neural activity, exposed metal recording sites are located in along the length of each of the needles (Figure 1 insert). These recording sites are routed along the needle and aggregated at the base of the array for further routing, and amplification and digitization off-chip. Our approach relies on a number of innovations in different parts of the system design. We developed a simple and customizable toolset to automatically generate the different design components needed, which we then fabricate and assemble into a three-dimensional array. Further, we created mechanical and electrical connections for the arrays as well as packaging solutions aimed at head-fixed neural recordings in rodents. Finally, we are using electron beam lithography to define sub-micron pitched but centimeter long metal wiring for the signal routing. This allows us to create very narrow needles that minimize tissue displacement, and enables us to achieve scalable high-density arrays.

Acetylcholine drives cortical microcircuit and modulates temporal dynamics in V1.

H. SUGIHARA, N. CHEN, M. SUR

Presented by: Hiroki Sugihara

Acetylcholine (ACh) modulates cortical functions including information processing and plasticity. To understand the physiological basis of these functions, it is critical to identify the cortical circuit elements involved. We have previously shown that cholinergic activation of astrocytes and their facilitatory influences on pyramidal neurons (PYR) are crucial to induce plasticity (Chen N, Sugihara H et al., PNAS 2012). In this work, we aim to dissect the neural circuit involved in cholinergic modulation of sensory processing. Specifically, we focus on the temporal dynamics of cortical activity: decorrelation of neuronal responses and desynchronization of local field potential (LFP) using L2/3 mouse primary visual cortex (V1) as a model. Recent studies suggest that inhibitory neurons are important for mediating temporal changes in neural activity. Candidate neurons include regular-spiking inhibitory neurons: somatostatin-expressing (SOM), vasoactive intestinal peptide-expressing (VIP) and layer 1 (L1) neurons. We recorded the cholinergic responses of these inhibitory neuronal subtypes in slice preparations. ACh induced concentration-specific responses in these neurons: SOM neurons were activated by a range of

ACh concentrations while VIP/L1 neurons were activated only at high concentration. We further show that this is likely due to the active shaping of inhibitory neuronal responses through defined inhibitory connections between them: SOM neurons inhibit VIP/L1 neurons and this counters the ACh-induced facilitatory responses in the VIP/L1 neurons. In addition, we show that ACh-activated SOM (but not VIP/L1) induced inhibitory currents in parvalbumin-expressing (PV) and PYR neurons. This suggests the presence of an ACh-activated neural circuit comprising direct SOM-PYR and indirect SOM-PV-PYR connections. We next tested the causal relationship between this SOM-driven circuit and decorrelation/desynchronization through hyperpolarizing Arch-expressing SOM neurons in vivo. Indeed, hyperpolarization of SOM neurons blocked the cholinergic-mediated desynchronization/decorrelation. Hyperpolarization of VIP neurons did not affect the LFP desynchronization. Finally, we stimulated SOM neurons directly by expressing ChR2 in these neurons. Photostimulation of SOM neurons, in the absence of cholinergic stimulation, induced LFP desynchronization. This suggests that direct activation of SOM-driven circuit is sufficient to change temporal dynamics of V1. Collectively, these findings reveal the powerful role of SOM neurons in dynamically shaping the temporal pattern of cholinergic-mediated responses.

Dys-regulation of hippocampal protein translation in a mouse model of Rett Syndrome
J. TAO, H. WU, A. A. CORONADO, E. de LAITTRE, R. W. KOMOROWSKI, Y. ZHANG, M. F. BEAR
Presented by: Jifang Tao

Rett Syndrome is a severe form of autism spectrum disorder caused by mutations in the gene encoding methyl-CpG binding protein 2 (MECP2). Using a *Mecp2 KO* mouse model that recapitulates a wide range of physiological and neurological abnormalities of human patients, we demonstrate that protein translation and metabotropic glutamate receptor 5 (mGluR5) dependent synaptic plasticity are altered in the hippocampus of *Mecp2 KO* mice at early symptomatic stage. Genome-wide profiling of translating mRNAs in wild-type and *Mecp2 KO* hippocampal CA1 neurons defines 'Mecp2-regulated translatome'. Compared to FMRP direct targets, cytoskeleton and neuron projection morphogenesis are two major groups of shared downstream targets between MeCP2 and FMRP, which may also be regulated by mGluR signaling. Furthermore chronic treatment with a negative allosteric modulator (NAM) of mGluR5 prolongs life span, reduces long breath holdings, partially rescues reduced hippocampal neuronal soma size and ameliorates inhibitory avoidance deficit in *Mecp2 KO* mice. These results indicate a potential link of transcriptional and translational regulation on a common set of genes by MeCP2 and FMRP.

The maternal IL-17a pathway promotes autism-like phenotypes in offspring
Y. S. YIM, H. WONG, S. LOM, H. KIM, S. V. KIM, C. A. HOEFFER, D. R. LITTMAN, J. R. HUH, G. B. CHOI
Presented by: Yeong Shin Yim

Human studies suggest that maternal viral infection during pregnancy correlates with an increased frequency of Autism Spectrum Disorder (ASD) in the offspring. This observation has been modeled in rodents subjected to maternal immune activation (MIA). The immune cell populations involved in induction of ASD-like behavior in the MIA model have not been identified. Using both genetic mutants and blocking antibodies, we show that RORγt- dependent effector T lymphocytes (e.g. Th17 cells) and the effector cytokine interleukin-17a (IL-17a) are required in mothers for MIA-induced behavioral abnormalities in the offspring. We find that MIA induces an abnormal cortical phenotype, which is also dependent on maternal IL-17a, in the fetal brain. Our data suggest that therapeutic targeting of Th17

cells in susceptible pregnant mothers may reduce the likelihood of bearing children with inflammation-induced ASD-like phenotypes.

Characterization of Glia Calcium Signaling in the Regulation of Neuronal Excitability
Y. V. ZHANG, J.T. LITTLETON
Presented by: Yao V. Zhang

Alterations in neuronal excitability can lead to severe neurological disorders like epilepsy. Studies in recent years have begun to reveal the essential role of glia in modulating the function of neural circuits. Previous work in our lab identified Zydeco (Zyd), a cortex glial sodium calcium potassium exchanger (NCKX), as a critical regulator of acute neural excitability in *Drosophila*. Mutations in *zyd* predispose flies to temperature-sensitive seizures and result in neuronal hyperexcitability at elevated temperatures. Meanwhile, near-membrane microdomain calcium transients found in wildtype cortex glia are abolished in *zyd* mutants, which instead show elevated intracellular glial calcium levels. These observations suggest a calcium-dependent cortex glia-to-neuron signaling cascade acutely regulates neuroexcitability in the *Drosophila* CNS. Characterizing this signaling cascade will greatly contribute to our understanding of how glia and neurons work in concert to maintain normal function of the nervous system. To further define this pathway, we performed an RNAi screen to knock down candidate genes in the *zyd* background using the pan-glial repo-Gal4 driver. Interestingly, we identified many seizure suppressors from the screen that were required in astrocyte-like glia instead of cortex glia, where the Zyd protein acts. This unexpected observation indicates that astrocyte-like glia function to propagate seizure-like activity that arises from a primary defect in cortex glia function. As such, both cortex glia and astrocyte-like glia play critical roles in regulating neuronal excitability in response to seizure-inducing signals arising from elevated calcium within cortex glia. We will present current progress in these studies in an effort to identify the mechanisms that distinct glia subtypes use to regulate neuronal function and excitability.