2013 SCSB Fall Poster Session Abstracts

Role of cortical interneuron subtypes in Rett syndrome

A. BANERJEE, R. RIKHYE, J. CASTRO, C. RUNYAN, M. SUR

Presented by: Abhishek Banerjee

Maturation of Parvalbumin (PV+) and Somatostatin (SOM+)-expressing interneuron-mediated inhibition plays an important role in shaping cortical plasticity and directly affects integration of sensory inputs and excitatoryinhibitory balance - features that are disturbed in Rett syndrome (RTT). RTT is a neurodevelopmental disorder of genetic origin, caused by mutations in the Xlinked gene methyl-CpG binding protein 2 (MeCP2). Effects of global loss of MeCP2 from forebrain excitatory and inhibitory neuronal population has previously been studied; however, little is known about how MeCP2 regulates the development and plasticity of specific subpopulations of GABAergic inhibitory circuits and how they are altered in RTT. To identify cortical circuit abnormalities that are specifically associated with MeCP2 deficiency, we have analysed the properties and role of PV+ and SOM+ expressing inhibitory interneurons, using mouse visual cortex (V1) as a model system. To study the role of MeCP2 in experience-dependent inhibitory circuit Maturation of Parvalbumin (PV+) and Somatostatin (SOM+)-expressing interneuron-mediated inhibition plays an important role in shaping cortical plasticity and directly affects integration of sensory inputs and excitatoryinhibitory balance - features that are disturbed in Rett syndrome (RTT). RTT is a neurodevelopmental disorder of genetic origin, caused by mutations in the Xlinked gene methyl-CpG binding protein 2 (MeCP2). Effects of global loss of MeCP2 from forebrain excitatory and inhibitory neuronal population has previously been studied; however, little is known about how MeCP2 regulates the development and plasticity of specific subpopulations of GABAergic inhibitory circuits and how they are altered in RTT. To identify cortical circuit abnormalities that are specifically associated with MeCP2 deficiency, we have analysed the properties and role of PV+ and SOM+ expressing inhibitory interneurons, using mouse visual cortex (V1) as a model system. To study the role of MeCP2 in experience-dependent inhibitory circuit maturation and OD plasticity, we specifically deleted MeCP2 from PV+ and SOM+ interneurons and compared the findings with global MeCP2 KO mice. Using optical imaging of intrinsic signals, ipsi- and contralateral eye-specific responses were recorded from layer 2/3 of V1 during postnatal day (P) 28-30. Deletion of MeCP2 did not significantly alter OD plasticity after 3-4 days of monocular deprivation. Optogenetic activation of MeCP2-deficient PV+ and SOM+ cells revealed no change in their inhibition profile on target cells, analyzed with two-photon guided in vivo loose-patch recordings. However,recordings from RFP+ MeCP2 deleted PV+ and SOM+ interneurons in layer 2/3 of mouse V1 revealed that visual response properties of these interneurons are significantly altered compared to control PV+ and SOM+ cells. Overall, we found that loss of MeCP2 in specific subsets of interneurons does not significantly alter plasticity during critical periods of mouse visual cortex development. Their inhibitory synaptic output also remains unaffected. However, there is a significant alteration of excitatory visual drive onto these interneuron subtypes. Taken together, these observations imply crucial cell-autonomous abnormalities in selective synapses and cortical circuits due to MeCP2 deficiency.

MULTI-VIEW NETWORK MODULE DETECTION

Y.-T. CHANG, D. PANTAZIS

Presented by: Yu-Teng Chang

We propose a novel method to solve the joint modular structure identification from multiple networks. The combination of modular detection and multi-view clustering provides a formal mathematical framework to jointly partition multiple networks formed from diverse real-life systems, such as fMRI connectome.

Asilomar Conference, 2013

REGULATION OF COMPLEXIN FUNCTION MODULATES ACTIVITY-DEPENDENT GROWTH OF DROSOPHILA SYNAPSES

R. W. CHO, L. BARR-BUHL, D. VOLFSON, J. T. LITTLETON

Presented by: Richard William Cho

The Drosophila neuromuscular junction (NMJ) is highly plastic and undergoes activity-dependent synaptic proliferation over the course of larval development. We have previously found that complexin (cpx) null mutants exhibit a dramatic increase in spontaneous action potential-independent synaptic vesicle release (minis) and a reduction in action potential-dependent fusion at Drosophila NMJs. This suggests a dual function for Cpx at the synapse -- preventing unregulated spontaneous fusion and facilitating action potential evoked release. This dual function of Cpx is genetically separable and is likely mediated by distinct molecular mechanisms. Here we show that Cpx is a substrate for PKA phosphorylation, which can modify its role in activity-dependent functional and structural plasticity. In-vivo experiments using cpx phospho-incompetent mutant rescue experiments in the cpx null background demonstrate that Cpx phosphorylation is required for activity-dependent structural plasticity at the Drosophila NMJ. We examined the functional consequences of Cpx phosphorylation using point mutants at specific Cpx phosphorylation sites, and find that its function as a clamp and facilitator of vesicle release is differentially modulated. It is well established that increased neuronal activity through action potential-evoked release promotes synaptic growth and functional changes, but the biological contributions of minis are less clear. We find that spontaneous vesicle fusion can also regulate synaptic growth through a retrograde signaling pathway. These data suggest that multiple signaling pathways may converge on Cpx to differentially regulate its synaptic function, and that this regulation is required for synaptic growth and activity-dependent plasticity.

Cold Spring Harbor Laboratories--Neurobiology of Drosophila October 2013

AUTOMATED IMAGE-GUIDED WHOLE-CELL PATCH CLAMP TECHNOLOGY FOR MAPPING FUNCTIONAL NEURONAL CIRCUIT CONNECTIVITY IN THE AUTISM SPCTRUM DISORDERS

A. A. CHUBYKIN, B. CALLAHAN, E. S. BOYDEN, M. F. BEAR

Presented by: Alexander A. Chubykin

A critical question in autism research is whether different genetic causes converge on a limited number of pathophysiological mechanisms. Of particular interest is the question of whether synaptic connectivity, function, and plasticity is at the core of autism pathophysiology. The tool best suited to address this question is the whole-cell patch clamp technique with which multiple aspects of excitatory and inhibitory synaptic currents, cellular excitability, and interneuronal connectivity can be characterized. However, this

method has extremely low throughput because of the level of expertise currently required of the experimenter to achieve good recordings from connected neurons. We have overcome this roadblock by developing a new image-guided Autopatcher 3D system for brain slices, extending the blind in vivo automated whole-cell patch clamp prototype developed in the Boyden laboratory. This system provides image-guided whole-cell patch clamp electrophysiology for analyzing functional neuronal connectivity in vitro, and can be combined with Channelrhodopsin-Assisted Circuit Mapping (CRACM) to map functional connectivity in different brain areas. We are currently using this system to test whether basic physiological mechanisms are altered in the excitatory or inhibitory neurons of the primary visual cortex and in primary neuronal cultures, and if these alterations underlie increased excitability and excessive persistent neuronal activity in the Fragile X mouse model.

The social epidemiology of Autism Spectrum Disorder: An examination of social and organizational risk factors for diagnosis

P. COLATAT, L. CROEN, E. ZUCKERMAN, S. SILBEY, S. DAWOOD

Presented by: Phech Colatat, Shadab Dawood

Organizational influence in autism diagnosis: Adaptation from imprinting

P. COLATAT

Presented by: Phech Colatat

What explains differences in ASD (autism spectrum disorder) diagnosis rates? Recent research has highlighted the role of social factors in ASD diagnosis but surprisingly has not examined the role of the central actor in ADS diagnosis: the healthcare provider. I study the diagnosis ASD in a large healthcare organization, Kaiser Permanente Northern California (KPNC), between 2000 and 2012. Rates of ASD diagnosis increased throughout the 1990s and 2000s at KPNC, consistent with broader changes in the field, but substantial variation was found across three specialized ASD clinics with diagnosis rates differing by as much as 30 percentage points. This is particularly surprising, considering all clinics are part of the same healthcare organization and should have the same diagnostic practices. In my research, I rule out the alternative explanations that these differences were driven by incentives, patient characteristics, health care provider characteristics, and referral patterns. Instead, I trace the difference in diagnostic outcomes to subtly distinct professional approaches at each clinic. These different approaches can be traced to the professional approaches of each clinic?s founding director and to the directors? educational experiences in ASD.

Restricted, Repetitive Behaviors and Specifically Correlated RNA Profiles Induced by D-Amphetamine

B. CHILINGIRIAN, H. BOWDEN, J. CRITTENDEN, D. HOUSMAN, A. GRAYBIEL

Presented by: Jill Crittenden, Hilary Bowden, Berj Chilingirian

Restricted repetitive behaviors that have no apparent utility are termed stereotypies and are associated with a variety of pharmacological, environmental, and genetic conditions including drug intoxication, stress, OCD and autism. Pathological stereotypies are associated with abnormalities in the cortico-striatal circuit that normally subserves motor learning and habit formation. Psychomotor stimulants such as amphetamine cause dopamine release in the striatum, which promotes the expression of stereotypies. To identify gene expression changes associated specifically with severe stereotypy, we first sought to define an amphetamine treatment regime that induces maximal sensitization of stereotypy. Observational and automated measurements of stereotypy were made across days in mice that were treated with d-amphetamine for 21 days. We found that mice undergo significant sensitization (augmentation) of their stereotypy response within the first few days of d-amphetamine treatment and develop a preferred cage location for engagement in stereotypy. Across the second two weeks of treatment, the duration of the confined stereotypy response diminished as locomotor responses predominated. We identified significant

RNA changes in the dorsal striatum of mice treated with the 7 day, stereotypy-sensitizing protocol by comparing changes among mice in six different treatment groups (d-amphetamine or vehicle for 1, 7 or 21 days). There was a predominance of changes in transcription factors, including Per, the nr4a family and NPAS family.

Exploring superior temporal sulcus responses and patterns with a broad set of naturalistic stimuli

B. DEEN, N. KANWISHER, R. SAXE

Presented by: Ben Deen

The superior temporal sulcus (STS) has been identified as a critical region for social perception. Prior experiments have used targeted contrasts (e.g., faces versus objects or human motion versus object motion) to investigate STS functional organization. However, the space of visual social stimuli is very broad, and experiments intended to isolate a single feature may miss other dimensions of relevance to the STS. Additionally, while most prior work on the STS has focused on responses of individual voxels or regions of interest, recent fMRI studies in other domains have demonstrated that multivoxel patterns of activity often carry more information than mean responses alone. In the present study, we measured STS responses to 282 dynamic social stimuli (3s-long film clips depicting humans acting and interacting), and related responses and patterns to a number of continuous social dimensions. These dimensions, defined by obtaining behavioral ratings of the stimuli, included various types of biological motion (head, hand, eye, and leg motion), the presence of talking, presence of a social interaction, strength of emotions depicted, and emotional valence. Low-level visual properties (luminance, contrast, and motion) were included as controls, and all measures were orthogonalized with respect to the others. We find that patterns in a common posterior STS region explain unique variance in a large number of social perceptual dimensions, including different types of biological motion, the presence of interactions and talking, and strength of emotions. Additionally, the presence of talking related to patterns in multiple regions along the full length of the STS. Methodologically, relating patterns rather than univariate responses to these stimulus dimensions improved sensitivity across the board.

Molecular tuning of Wnt signaling during neuronal development by Autism-associated gene CHD8

O. DURAK, Y. J. KAESER-WOO, L.-H. TSAI

Presented by: Omer Durak, Yea Jin Kaeser-Woo

Autism spectrum disorders (ASDs) represent an etiologically heterogeneous group of developmental disorders of the human brain. Our goal is to dissect the molecular pathways that cause ASDs. The Wnt signaling pathway is critical for the formation of the nervous system: it promotes neuronal proliferation and migration in the developing brain. Chromodomain Helicase DNA-binding 8 (CHD8), an important regulator of Wnt pathway, was first associated with cognitive impairment and recent whole-exome sequencing of de novo mutations in sporadic ASD has further strengthened the evidence for CHD8 as a major risk gene of ASD. Our initial observations of developing mouse brain suggested that depletion of CHD8 using RNA interference results in a series of developmental deficiency including dysregulation of neuronal proliferation as well as migration and dendritic branching of newborn neurons. In combination of luciferase reporter assay and biochemical characterization of CHD8, we are beginning to understand the mechanism by which CHD8 regulates Wnt pathway during neuronal development. These observations will provide insight into the mechanism contributing to ASD.

High-content in vivo analysis of neuronal activity and patterning in zebrafish

P. M. EIMON, A. ALLAOU, L. FREIFELD, M. REZAIE, Y. WU, M. F. YANIK

Presented by: Peter M. Eimon, Limor Freifeld, Mostafa Rezaie

The zebrafish (Danio rerio) is a powerful model system for high-throughput in vivo characterization of genes implicated in autism spectrum disorders (ASD). Genome wide association studies and exome

sequencing have identified hundreds of rare alleles, copy number variants, and de novo mutations associated with ASD. Validating the biological significance of these mutations is challenging due to the complex developmental and cognitive deficits underlying autism, which cannot be easily modeled in cell culture assays. Our lab is developing two complimentary approaches for high-throughput/high-content imaging of neurological phenotypes that arise during CNS development in zebrafish larvae. 1) An automated optical projection tomography platform to image brain patterning in three dimensions at near-cellular resolution throughout early development. 2) Confocal and light-sheet microscopy platforms for real-time whole-brain imaging of neuronal activity in transgenic zebrafish expressing genetically encoded calcium indicators and optogenetic tools. Both approaches yield multidimensional quantitative datasets that allow highly complex neurological phenotypes to be measured, classified, and studied in unprecedented detail. Used in combination, these platforms will allow zebrafish mutants to be assessed for both morphological and functional deficits and may ultimately enable high-throughput drug screening to identify therapeutic compounds.

An isogenic human induced pluripotent stem cell model of Rett Syndrome reveals novel alterations in microRNA expression and downstream molecular targets.

N. MELLIOS, S. D. SHERIDAN, S. KWOK, D. FELDMAN, B. ROSEN, B. CRAWFORD, S. HAGGARTY, M. SUR

Presented by: Danielle Feldman

Rett Syndrome (RTT) is an X-linked monogenic form of autism spectrum disorder that is predominantly caused by mutations in methyl CpG-binding protein 2 (MECP2). Clinical features of RTT include a period of normal development lasting up to 6-18 months followed by stagnation of both neurological and general growth development. As this is a disorder of early development, it has proven difficult to elucidate phenotypes and/or molecular signatures at a pre-symptomatic stage. We have generated human induced pluripotent stem cell (iPSC) lines in which to study molecular and functional phenotypes in vitro at various stages of neural development. By utilizing x-inactivation, we are able to generate isogenic cell lines that originate from the same patient and differ exclusively at the disease allele. Additionally, we have knocked down MECP2 expression in healthy control cell lines using viral shRNA constructs. Using dual-SMAD inhibition, we induce iPSCs to adopt a neural lineage. Subsequently, we differentiate these neural progenitors into cortical neurons. iPSC-derived neurons are capable of firing action potentials and exhibit spontaneous synaptic activity, observed via whole-cell patch clamp and functional calcium imaging. Using these isogenic patient-derived and MECP2 knockdown cell lines, we screened for and validated miRNAs altered in RTT at both neural progenitor and neuronal stages. Results identified a miRNA family that is robustly augmented in RTT at both time points in patient-derived cell lines, as well as in control cell lines following MECP2 knockdown via shRNA. In parallel, components of the mitogen-activated protein kinase (MAPK) pathway that are known targets of the upregulated miRNA family were found to be misregulated in RTT lines. RTT lines exhibit a phenotypic delay in maturation, as seen in morphological and protein expression-based analyses. Ongoing experiments are focused on elucidating the mechanisms that underlie these deficits, particularly as they relate to the aberrant signaling regulation at hand. As such, we?ve shown that MeCP2-mediated miRNA expression is altered in a human model of RTT and has significant effects on downstream signaling pathways implicated in RTT pathogenesis.

Amygdala inputs to the ventral hippocampus bidirectionally modulate social behavior

A. C. FÂ • LIX-ORTIZ, K. M. TYE

Presented by: Ada Felix-Ortiz, Neha Bhagat

Impairments in social interaction represent a core symptom in a number of psychiatric disease states including autism, schizophrenia, depression and anxiety. Although the amygdala has long been linked to social interaction, little is known about the functional role of connections between the amygdala and downstream regions in non-competitive social behavior. In the present study, we used optogenetic and pharmacological tools in mice to study the role of projections from the basolateral amygdala (BLA) to the

ventral hippocampus (vHPC) during two social interaction tests; the resident- juvenile intruder paradigm and the three chamber sociability test. BLA pyramidal neurons were transduced using adeno-associated viral vectors (AAV5) containing either channelrhodopsin-2 (ChR2) or halorhodopsin (NpHR), under the control of the CaMKIIÃ promoter to allow for optical excitation or inhibition of amygdala axon terminals. Optical fibers were chronically implanted to selectively manipulate BLA terminals in the vHPC. NpHR-mediated inhibition of BLA-vHPC projections significantly increased social interaction on the resident-juvenile intruder paradigm as shown by increased intruder exploration. In contrast, ChR2-mediated activation of BLA-vHPC projections significantly reduced social behaviors as shown in the resident-juvenile intruder paradigm by decreased time exploring the intruder and in the three chamber sociability test by decreased time spent in the social zone. These results indicate that BLA inputs to the vHPC are capable of modulating social behaviors in a bidirectional manner.

Automated phenotyping of mouse behavior

C. WANG, Y. NI, S. BRAUN, N. EDELMAN, H. JHUANG, G. FENG, T. POGGIO

Presented by: Charlie Frogner

We have developed a trainable computer vision system that automates the analysis of complex mouse behaviors, for a single mouse in its home cage [3]. Our system performs on par with human scoring, as measured from ground-truth manual annotations of thousands of clips of freely behaving mice. As a validation of the system, we characterized the home-cage behaviors of two standard inbred and two non-standard mouse strains. From these data, we were able to predict in a blind test the strain identity of individual animals with high accuracy.

Moving beyond single-mouse behaviors, we have also developed a system for tracking multiple mice throughout their social interactions [4,5,6]. Our system performs with high accuracy against human scoring of mouse trajectories and pose, and is able to maintain the identities of the mice through complex physical interactions. We are currently applying this system to analyze the social behaviors of a number of transgenic models, with the hope of gaining additional quantitative insights into the social deficits associated with autism.

1. J. N. Crawley. Mouse behavioral assays relevant to the symptoms of autism. Brain Pathology, 17:448-459, 2007.

2. J. Peca. C. Feliciano. J. Ting. W. Wang. M. Wells. T. Venkatramann. C. Lascola. Z. Fu. and G. Feng. Shank3 mutant mice display autistic-like behaaviors and striatal dysfunction. Nature, 2011.

3. H. Jhuang, E. Garrote, X. Yu, V. Khilani, T. Poggio, A. Steele, and T. Serre. Automated home-cage behavioral phenotyping of mice. Nature Communications, 2010.

4. N. Edelman. Automated phenotyping of mouse social behavior. M.Eng. thesis, MIT, 2011.

5. S. Braun. Tracking multiple mice. M.Eng. thesis, MIT, 2012.

6. C. K. Wang. Multiple mice tracking using Microsoft's Kinect. M.Eng thesis, MIT, 2013.

Sensory Hypersensitivities in Autism

T. K. GANDHI K. TSOURIDES, N. SINGHAL, D. PANTAZIS, M. KJELGAARD, P. SINHA

Presented by: Tapan K. Gandhi, Kleovoulos Tsourides

Sensory hypersensitivities are diagnostic of autism spectrum disorder (ASD). Estimates are that 88% of individuals with ASD experience sensory hypersensitivities, the most common being auditory. We posit that impaired neural habituation may be a causal factor. Habituation serves the function of adapting to predictable sensory stimuli such that cognitive resources are free to process new information.

Impairments in habituation to sensory stimuli would make for a confusing situation in which all sensory elements would be treated as equally salient and unpredictable. If one?s ability to discount even highly predictable sensory streams were impaired, the overwhelming sensory bombardment may manifest as a need to avoid further stimulation.

We studied habituation to auditory stimuli in children and young adults with ASD using two distinct measures of sensory sensitivity, one peripheral, galvanic skin response (GSR) and one central, magneto-

encephalography (MEG). Both these assessment techniques reveal reduced sensory habituation in ASD individuals relative to NT controls.

Understanding of social agency in real scenes.
T. GAO, J. TENENBAUM, N. KANWISHER
Presented by: Tao Gao
Social agents' behaviors are governed by their mental states, including goals, intentions, beliefs, and desires. Understanding of others' mental states is the foundation of various types of social interaction.Developmental studies have demonstrated how infants? or children's social interactions with adults can be altered by their understanding of the adults' intentions. In contrast, we know little about how to build a formal model that can describe the computational processes involved in such rich social understandings. The difficulty of building such a model is in part due to the fact that extracting and representing human actions involve intensive image processing and sophisticated motion planning algorithms, which are typically outside the scope of developmental studies. Here we propose an interdisciplinary approach for this topic by combining theories from developmental psychology, psychophysical methods from cognitive psychology, and state-of-the-art techniques from computer vision and robotics. In particular, we describe (1) how to extract human movements and the 3D representation of the environment with the Kinect sensor; (2) how to measure the percepts of human movements with rigorous psychophysics; and (3) how to build a computational model of such percepts, motivated by developmental studies related to theory of mind; (4) how to implement such a model by taking advantage of the latest motion planning techniques from robotics.

Awake in vivo Ca2+ responses in astrocytic processes of mouse visual cortex.
R. I. GARCIA, M. GOARD, J. PETRAVICZ, M. SUR
Presented by: Rodrigo Garcia
Astrocytic intracellular Ca2+ signaling has become a prominent feature in neuronal-glial interactions. The majority of data concerning astrocyte Ca2+ signaling come from either culture or in situ brain slices, an approach that relies on electrical stimulation or pharmacological methods to examine the spatial and temporal coding of astrocyte Ca2+ signals. Recently, several studies have utilized in vivo Ca2+ imaging in response to physiologically relevant stimuli or combined with electrical stimulation of brain nuclei to examine the role of astrocyte Ca2+ transients in intact circuits. Further, in situ data shows that localized Ca2+ elevations in distal processes of astrocytes occur at a higher frequency than somatic increases. This is difficult to examine in vivo; bulk loading of SR101 and OGB require anesthesia and allow for imaging primarily of somatic response, with limited detection of Ca2+ activity in processes. Additionally, anesthesia has been shown to influence both neuronal and astrocytic activity, which may alter the spatial and temporal coding of Ca2+ transients in response to stimuli. We are currently investigating visually evoked Ca2+ responses in visual cortex astrocytes of an awake, head-fixed animal using two-photon microscopy. To achieve this, we have generated a new astrocyte reporter line that expresses tdTomato in cortical astrocytes driven by the shortened human GFAP promoter, together with viral mediated delivery of a membrane-bound genetically encoded Ca2+ indicator, Lck-GCamp5G, to specifically target cortical astrocytes. We present evidence that Ca2+ transients in distal processes of cortical astrocytes are more frequent than has been observed for anesthetized preparation, and are not correlated to somatic responses. Furthermore, we are able to identify structurally identifiable regions of distal processes from single astrocytes that are responsive to visual stimulus and display orientation tuning; continued experiments will determine if this will reflect the tuning of surrounding neurons. The combination of these technologies will allow us to further explore the functional role of astrocytes in the primary visual cortical circuit.
SfN 2013

A genetic screen for regulators of Synaptotagmin 4-dependent postsynaptic exocytosis

K. P. HARRIS, Z. D. PICCIOLI,N. PERRIMON, J. T. LITTLETON
Presented by: Katie Harris
Postsynaptic cells can induce synaptic plasticity through the release of retrograde signals in response to presynaptic activity. We have previously identified a calcium-dependent retrograde signaling pathway that is mediated by postsynaptic Synaptotagmin 4 (Syt4) at Drosophila neuromuscular junctions (NMJs). We hypothesize that Syt4 acts as a Ca2+ sensor to control the release of postsynaptic retrograde signals, similar to the role of Syt1 in presynaptic vesicle fusion. However, little is known about how postsynaptic exocytosis is regulated and what cargo molecules are trafficked in the Syt4 pathway. Further analysis of Syt4-mediated signaling will enhance our understanding of how synaptic growth and plasticity are regulated.
To identify genes involved in Syt4 trafficking, we assembled a UAS-RNAi transgenic collection targeting 450 gene products that are resident at synapses and/or putatively involved in membrane trafficking. Our screening approach employed transgenic animals expressing Syt4 tagged with pHluorin, a pH-sensitive variant of GFP (UAS-Syt4-pH), allowing for in vivo visualization of exocytosis of Syt4 positive vesicles. When expressed in muscle, UAS-Syt4-pH decorates the postsynaptic membrane of the NMJ, overlapping with glutamate receptor (GluR) fields opposite active zones. Candidate UAS-RNAi constructs were co-expressed with UAS-Syt4-pH in muscle, and animals were examined for changes in Syt4-pH distribution. We identified several candidates that regulate Syt4-pH distribution at the NMJ, including the t-SNARE Syntaxin 4 (Syx4). RNAi knockdown of Syx4 results in a reduction in Syt4-pH levels at the postsynaptic membrane and abnormal accumulation of Syt4-pH in the cytoplasm. To further explore the function of Syx4, we generated loss-of-function mutants. These animals exhibit a reduction in the number of boutons at the NMJ, indicating impairment of synaptic growth. Syx4 protein is enriched postsynaptically at the NMJ. These observations are consistent with a role for Syx4 as a t-SNARE for postsynaptic exocytosis. Current studies are examining synaptic function and structure in Syx4 mutant animals. In addition, the RNAi screening approach should help identify additional regulators of postsynaptic exocytosis and retrograde signaling, expanding our understanding of synaptic communication and neuronal plasticity.
Neurobiology of Drosophila, Cold Spring Harbor, October 1-5 2013

Examining de novo mutation rates and patterns from whole-exome sequencing of sporadic cases of Schizophrenia from Taiwan
D. HOWRIGAN, B. NEALE, J. MORAN, K. CHAMBERT, S. ROSE, N. LAIRD, H.-G. HWU, W.-J. CHEN, C.-M. LIU, C.-C. LIU, J. NEMESH, E. BEVILACQUA, A. HANSEN, S. V. FARAONE, S. GLATT, M. TSUANG, S. MCCARROLL
Presented by: Daniel Howrigan
Our primary aim in this study is to identify specific genes that, when disrupted by a de novo mutation, lead to sporadic cases of schizophrenia. Whole-exome sequencing has been performed on 1,135 complete trios, making it one of the largest de novo sequencing projects to date. We are currently halfway through Illumina MiSeq validation of over 1,800 identified de novo single nucleotide variants and indels, with 1,157 of these being stringent quality calls with > 20 reads in each member of the trio. Among the validated and stringent quality calls, a per-exome de novo mutation rate of 1.03 is observed, in line with expectation and published reports of de novo mutations in schizophrenia. This rate will change only slightly upon full validation, as we have observed a 90% validation rate of our putative de novo targets to date. In our ongoing analyses, we have validated two loss-of-function de novo mutations disrupting synaptic vesicle glycoprotein 2B gene (SV2B), however among this large trio set, this observation does not surpass exome-wide significance (p=2.18e-5). Among the genes containing at least one loss-of-function and/or missense mutations, we see an enrichment among genes implicated in the Fragile X RNA-Binding protein network (p=3.85e-3 for loss-of-function and p=.027 for missense). This network comprises an integral list of binding targets for neuronal function within the brain (Darnell et al., 2011 and Darnell et al., 2013), and has also been implicated in de novo studies of autism (Iossifov et al., 2012) and intellectual disability.

Functional analysis of command neurons for feeding behavior in Drosophila

S. IGUCHI, M. YOSHIHARA
Presented by: Shinya Iguchi

Functional analysis of command neurons for feeding behavior in Drosophila

The decision of when to eat is a complex function of both environmental variables and internal physiological state. How these external and internal determinants are integrated by the nervous system is largely unknown, and the neural substrates of the feeding decision remain poorly characterized. Recent work from our lab, in collaboration with Ben White and Kei Ito, has identified a single pair of neurons in theDrosophilabrain, Fdg (feeding) neurons, which command the entire sequence of feeding behavior1. They were identified from a screen of Gal4 lines established by the NP consortium2 by activation of a neuron subset expressing a heat-activated cation channel, TrpA1. We used infrared laser light from a 2-photon microscope to spatially restrict heat to the cell body of either the left or right Fdg neuron expressing TrpA1, which led to asymmetric proboscis extension in the same direction as the illuminated side. Activation of a single Fdg neuron triggered pharyngeal pump movement mimicking natural feeding behavior. We used a strong illumination from a 2-photon laser to ablate the Fdg neuron by targeting the cell body. Ablation of an Fdg neuron cell body on one side made sucrose-induced proboscis extension response asymmetric, extending in the opposite direction. Furthermore, ablation of Fdg neurons on both sides led to complete suppression of feeding behavior, indicating a pivotal role for Fdg neurons in the feeding circuit. Here we examined how metabolic information is integrated in the feeding behavior circuit. Taking advantage of our newly devised method to record calcium signals while simultaneously observing feeding behavior3, we tested the effect of satiety on Fdg neuron activity correlated with feeding behavior. Fdg neurons were activated in response to food presentation and this coincided with a proboscis extension, but only in the starved state. We then tested the effect of starvation on TrpA1-induced proboscis extension, and observed increased frequency in proboscis extension in starved flies compared to satiated flies. Hyperpolarizing Fdg neuron by expressing Kir channel suppressed feeding behavior. However, stronger starvation neutralized the effect of hyperpolarization produced by Kir channel and rescued feeding behavior, supporting the possibility that starvation signal may depolarize Fdg neuron to enhance feeding behavior. These results support our hypothesis that Fdg neurons integrate starvation signal to make a decision of feeding behavior. 1) Flood et. al. (2013)Nature, 499, 83-87. 2) Yoshihara and Ito (2000)Drosophila Information Service. 83:199. 3) Yoshihara (2012)JoVE, 62, e3625, doi: 10.3791/3625.
2013 Cold Spring Harbor Laboratory - Neurobiology of Drosophila

Genetically engineered human pluripotent stem cell model of Rett Syndrome

Y. Li, H. WANG, J. MUFFAT, A. W. CHENG, D. A. ORLANDO, J. LOVEN, S.-M. KWOK, D. A. FELDMAN, H. S. BATEUP, Q. GAO, D. HOCKEMEYER, M. MITALIPOVA, C. A. LEWIS, M. G. VANDER HEIDEN, M. SUR, R. A. YOUNG, R. JAENISCH
Presented by: Yun Li

Rett Syndrome (RTT) is caused by mutations of MECP2, a methyl CpG binding protein thought to act as a global transcriptional repressor. Here we show, using an isogenic human embryonic stem cell (ESC) model of RTT, that MECP2 mutant neurons display key molecular and cellular features of this disorder. Unbiased global transcriptional analyses show that MECP2 acts as a global gene expression activator in neurons. Decreased transcription in mutant neurons was coupled with a significant reduction in nascent protein synthesis and lack of MECP2 was manifested as a severe defect in the ctivity of the AKT/mTOR pathway. Lack of MECP2 also leads to impaired mitochondrial function in mutant neurons. Activation of AKT/mTOR signaling by exogenous growth factors or by depleting PTEN oosted protein synthesis and

ameliorated disease phenotypes in mutant neurons. Our findings indicate a vital function for MECP2 in maintaining active gene transcription in human neuronal cells.
SFN 2013

Using multi-electrode array (MEA) to probe neuronal network activity defects in a dish
C. LU, T. ZHOU, Q. CHEN, Z. FU, G. FENG, J. PAN
Presented by: Congyi Lu
Dissociated neurons in culture retain many of the properties found in their in vivo context and are known to generate rich repertoire of spontaneous spiking activity which can be detected by multi-electrode array (MEA). Here, using MEA, we examined the role of Shank3, a postsynaptic protein whose disruption at genetic level associated with autism spectrum disorders (ASDs), in regulating neuronal network activity of cortical culture. We followed primary cortical cultures along developmental ages (from 7 to 21DIVs) and found that, compared to wild-type cultures, Shank3-/- cultures showed less spontaneous spiking activity, which became statistically significant at DIV21. In addition, after two weeks in culture, the overall network firing patterns for Shank3-/- networks was distinct from those of wild-type networks at the same DIV. We used various parameters to characterize network firing pattern including array wide spike detection rate (ASDR), active period, quiescent period, ASDR rise and decay kinetics. We found that wild-type networks displayed reliable firing patterns alternating between long active and long quiescent periods at DIV21, whereas most Shank3-/- network showed rapidly recycling between much shorter active and quiescent periods. This difference in network firing pattern suggested a phenotypic defect in network activity in the absence of Shank3 gene. We are further developing the assay to allow higher throughput screening, and ultimately to search for novel targets and mechanisms for developing treatment for ASDs using MEA phenotypic assays.
Symposium on the emerging genetics and neurobiology of severe mental illness, September 2013
Portion was presented at SfN 2012

Dissecting the role of the dorsal raphe dopamine neurons in social behavior
G. A. MATTHEWS, M. A. UNGLESS, K. M. TYE
Presented by: Gillian A Matthews
Impaired social interaction is a defining feature of autism spectrum disorders (ASDs), yet the underlying cause of this behavior remains unknown, and there are no established pharmacological treatments. Dopamine plays a central role in regulating behavior, and has been linked to a range of neuropsychiatric disorders including schizophrenia, depression, attention deficit hyperactivity disorder (ADHD), and ASDs. The most frequently studied midbrain dopamine neurons are those located within the substantia nigra and ventral tegmental area (VTA). However, a lesser known and understudied population of dopamine neurons reside within the dorsal raphe nucleus (DRN) and ventrolateral periaqueductal grey (vlPAG). Several lines of evidence suggest these neurons are anatomically and functionally distinct from dopamine neurons of the VTA. My data suggests that this subgroup may play a role in social interaction. I have found that DRN/vlPAG, but not VTA, dopamine neurons show an increase in the AMPAR:NMDAR ratio after just 24 hours of social isolation. Importantly, this potentiation appears to be dependent on previous social interaction, so that greater potentiation is observed in animals which have been removed from a larger social group. In order to further investigate this phenomenon I have begun to probe the glutamatergic input to DRN/vlPAG dopamine neurons, using retrograde tracing and optogenetics, in order to establish their role in social behavior. I ultimately plan to determine which input(s) are potentiated following social isolation, and to use this to modulate social behavior in vivo. Dissecting the neural circuits that underlie social interaction is a critical first step in understanding how and why deficits in social behavior emerge in ASDs.

Novel microRNA-mediated mechanisms regulating autism-related genes in Rett Syndrome - Implications for therapeutics.

N. MELLIOS, S. SHERIDAN, S. KWOK, D. FELDMAN, B. CRAWFORD, J. WOODSON, S. HAGGARTY, M. SUR
Presented by: Nikolaos Mellios
Rett Syndrome is a debilitating childhood-onset neurodevelopmental disorder that is predominantly caused by mutations in methyl-CpG-binding protein 2 (MEPC2). Using the MeCP2 knockout (KO) mouse model of the disease we uncovered a novel miRNA-mediated molecular pathway that bridges the observed alterations in Brain-derived neurotrophic factor (BDNF) and Insulin-like growth factor 1 (IGF1) expression in the brain of MeCP2 KO mice. Importantly, chronic treatment with a ?-2 adrenergic receptor agonist completely normalized the expression of the components of the affected molecular pathway in the cerebellum of Mecp2 KO mice, and resulted in greatly increased survival, improved respiratory function, social recognition, and motor coordination; all cardinal symptoms of Rett syndrome. Notably, coadministration of the same ?-2 adrenergic receptor agonist with recombinant human IGF1, further ameliorated the phenotype of MeCP2 KO mice, and resulted in a notable increase in survival. In parallel we used patient-derived induced pluripotent stem cells (IPSCs) to screen more effectively for miRNAs that are affected in Rett Syndrome. Our results from IPSC-derived neuronal cultures revealed among others an additional miRNA family that is robustly increased in two different patient-derived samples, at two different developmental stages, and following viral-mediated knockdown of WT samples. Protein and RNA expression analysis uncovered altered levels of known targets of the affected miRNA family, which are upstream regulators of BDNF expression. In summary we show using both a mouse and IPSC-model of Rett syndrome that a subset of Mecp2-regulated miRNAs are important effectors of complex regulatory networks related to brain growth factor expression, and reveal novel therapeutic alternatives for the treatment of Rett syndrome.
2013 Society for Neuroscience Conference - San Diego

Differences between dorso-lateral and dorso-medial striatum parvalbumin-interneurons
P. MONTEIRO, Y. ZHOU, I. R. WICKERSHAM, H. S. SEUNG, G. FENG
Presented by: Patricia Monteiro
The rodent brain displays a medial-lateral gradient of connectivity between cortex and striatum, resembling the connectivity observed in primates. In rodents, the dorso-medial striatum (DMS) could be compared to the Caudate and the dorso-lateral striatum (DLS) to Putamen. These spatially segregated regions seem to play complementary functional roles in the cortico-basal ganglia circuitry. Dorso-medial region has been shown to sustain goal-directed behavior whereas dorso-lateral region is mainly involved in habit formation. In perspective of microcircuitry, it is not clear how this is controlled. Striatum is mainly composed of medium spiny neurons (MSNs) orchestrated by local interneurons, including parvalbumin-positive fast-spiking interneurons (FSi). These FSi are thought to act as powerful striatum modulators, capable of spindle generation and control of large neuronal assemblies. Taking advantage of genetically engineered mice that label parvalbumin (PV) cells in the striatum, we find that PV interneurons located in dorso-lateral and dorso-medial striatum have distinct electrophysiological prints. A closer look to these cells reveals that not only they are distinctly connected (mEPSCs and mIPSCs) but also have different properties, with dorso-medial FSi being more excitable.
To further address the differences of these two groups of FSi, monosynaptic retrograde tracing through modified rabies virus is being used to map axonal inputs onto these two distinct interneuron populations. Understanding the physiology and connectivity of striatal interneurons will allow us to further dissect their roles in regulating striatal circuitry function and behavior.
"Champalimaud Neuroscience Symposium 2013 - Lisbon, Portugal"

Principles of high-fidelity, high-density 3-d neural recording
C. MOORE-KOCHLACS, J. SCHOLVIN, J. P. KINNEY, J. G. BERNSTEIN, Y. G. YOON, S. K. ARFIN, N. KOPELL, E. S. BOYDEN

Presented by: Caroline Moore-Kochlacs

What are the possibilities and limitations of neural recording techniques? How accurately can we resolve signals from individual neurons and how many neural signals can we record simultaneously? Previously, we used simulations to show that spike sorting with no spike assignment errors on a multielectrode array is possible, using an ICA-based spike sorting algorithm, but the utility of this approach was limited by yield. Only a small number of neurons were extracted in those simulations with probe layouts similar to existing multi-electrode recording probes. New probe designs, with an increased number of pads and decreased pad pitch, were shown to be promising directions for increasing the number of extracted neurons. Here we expand on that work and ask what probe design and layout would be required to record from a given brain volume, using the same spike sorting algorithm. Our simulations show that by increasing the pad density on a probe with fixed depth and width, the distance over which we can extract 100% of the neurons increases, to some saturation limit, after which increasing density ceases to increase the distance over which neurons are extracted at 100%. We performed robustness testing on the simulations to confirm this effect over a range of physiologically reasonable parameters. Across parameters, we consistently found that increased pad density led to increased distance over which 100% of neurons were extracted until the saturation limit. The distance and density for this saturation limit varied based these parameters. Some parameters that set these values, for example how the amplitude of a spike varies based on the detector it was recorded on, are sparsely characterized and must be directly measured to determine ideal probe designs. We are now beginning to test these ideas using custom high-density 3-D microelectrode designs.

SFN 2013

Predicting longer-term developmental outcomes from infants' exploratory behaviors

P. MUENTENER, E. HERRIG, L. SCHULZ

Presented by: Paul Muentener

How can we best identify the roots of atypical development early in infancy? Research suggests that basic measures of exploration (e.g., rate of habituation, degree of novelty preference) are better predictors of IQ than standardized developmental assessments. This raises the possibility that more sophisticated measures of exploratory behavior may have even greater predictive power for long-term developmental outcomes. I will present findings from a 4-year longitudinal investigation of infants? exploration and learning. We have found that measuring individual differences in infants? object exploration may be successful in predicting both short-term and longer-term developmental outcomes. These findings have implications for efforts at increasing detection of atypical development early in infancy.

The evolutionarily conserved protein VESA-1 controls synaptic vesicle acidification, synaptic function and behavioral state

N. PAQUIN, Y. MURATA, A. FROEHLICH, D. T. OMURA, C. PENDER, M. CONSTANTINE-PATON, H. R. HORVITZ

Presented by: Nicolas Paquin, Yasunobu Murata

The evolutionarily conserved protein VESA-1 controls synaptic vesicle acidification, synaptic function and behavioral state

Synaptic transmission is key to nervous system function and is essential as animals receive, store and integrate signals from their environment and their experience to modulate their behavioral responses. Defects in synaptic function have been associated with a variety of brain disorders. We discovered a novel synaptic protein, VESA-1, from studies of the behavior of the nematode Caenorhabditis elegans. VESA-1 controls worm behavioral state in response to past feeding experience. VESA-1 is evolutionarily conserved, and both VESA-1 and its murine homolog mVESA1 are expressed in neurons, associated with synaptic vesicles and control vesicle acidification, likely through modulation of a protein complex that

transports protons across the membrane of synaptic vesicles, the V-ATPase complex. We found that by regulating synaptic vesicle acidification, VESA-1 affects neuropeptide processing and neuropeptide release at synapses. Neuropeptides have been associated with the switch between two behavioral states. Interestingly, a deletion spanning the human VESA1 gene and only one other gene has been reported in an autistic patient, and it is possible that VESA-1 dysfunction is related to autism spectrum disorders. By further analyzing VESA-1 in C. elegans and mice, we hope to discover new molecules and new pathways fundamental to nervous system function and to better understand basic neurobiological processes and animal behavior as well as processes involved in neurological and neuropsychiatric disorders.

Mechanisms underlying rapid activity-dependent plasticity at Drosophila NMJs

Z. D. PICCIOLI, J. T. LITTLETON

Presented by: Zach Piccioli

Drosophila neuromuscular junctions (NMJs) are able to rapidly form new synaptic connections in response to elevated levels of neuronal activity in the form of incompletely developed synaptic boutons. These boutons are referred to as ghost boutons, though the mechanisms underlying rapid activity-dependent ghost bouton budding are not well understood. We have found that ghost bouton budding requires Ca2+ and normal glutamate receptor expression, but does not require an axonal connection to motorneuron cell bodies. Additionally, bouton budding requires local developmental retrograde BMP signaling. We have also identified a role for actin cytoskeleton regulation in bouton budding. Budding frequency is regulated by the activity level of Cofilin (Drosophila twinsar) and its regulator, LIM domain kinase (Limk). Live imaging of fluorescently labeled F-actin reveals that ghost bouton budding is accompanied by local formation of F-actin puncta. Pharmacological inhibition of actin turnover inhibits bouton budding. We are currently exploring the role of the type-II BMP receptor-Limk interaction on ghost bouton formation.
Cold Spring Harbor Neurobiology of Drosophila 2013

Neural signatures of phonological working memory and grammatical processing in autism spectrum disorders.

Z. QI, T. PERRACHIONE, A. HARRIS, I. OSTROVSKAYA, S. BEACH, K. HALVERSON, A. CYR, K. SHER, M. KJELGAARD, J. GABRIELI, K. WEXLER, T. TAGER-FLUSBERG

Presented by: Zhenghan Qi

Language deficits are one of the core impairments of autism spectrum disorders (ASD). Behavioral studies have documented reduced phonological working memory capacity and impaired grammatical processing in children with ASD (Kjelgaard & Tager-Flusberg, 2001). The current study is the first to probe the neural characteristics of these two key language functions in children with ASD and their typically developing (TD) counterparts. Participants were all native English-speaking children and subject groups were matched on age, non-verbal IQ, and performance on standardized language tasks (Clinical Evaluation of Language Fundamentals, Fourth Edition).
In Experiment 1, 22 children (11 ASD and 11 TD) completed a non-word repetition task during fMRI. Stimuli were pseudowords designed to match the phonological and phonotactic properties as real English words and ranged from two to five syllables in length. As predicted, the ASD group performed significantly worse than the TD group in overall accuracy (78% vs. 87%, $p < .05$). Imaging results revealed that repeating nonwords activated bilateral superior temporal gyri and left prefrontal areas in both groups, but that children with ASD showed a significantly reduced task-induced activation in the right temporal pole (FDR = 0.05, $p < .005$) as compared to TD children. As syllable length increased, both groups exhibited decreasing accuracy in performance ($r = 0.14$, $p < .05$). Compared with TD, children with ASD decreased the recruitment in right temporal pole as syllabic length increased (FDR = 0.05, $p < .005$).
In Experiment 2, 32 children (16 ASD and 16 TD) completed an auditory grammaticality judgment task during fMRI. Children listened to short sentences, which were either grammatically correct or contained

morphosyntactic errors and decided if each sentence sounded correct or not. The type of errors was characteristic of those made by TD during initial language acquisition and children with language impairments for a protracted length of time (e.g. Every day he walk to school). As compared to the TD group, the ASD group performed marginally worse (89% vs. 95%, $p = .076$) on this task, and had significantly reduced task-induced activation in left prefrontal cortex and left anterior cingulate cortex (FDR = 0.05, $p < .005$). The activation in left prefrontal cortex is negatively correlated with autism severity ($r = -.41$, $p < .05$).
These preliminary results revealed an atypical neural profiles for children with ASD as compared to typically developing children in fundamental language functions. Future research with larger sample size will compare the developmental trajectory of the neural recruitment across various age groups between typical developing children and those with ASD.
5th Neurobiology of Language Conference & 43rd Annual Meeting of the Society for Neuroscience, San Diego, CA

Tools for 3-D Brain Activity Mapping in Autism Models

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

Presented by: Jorg Scholvin

There are several hundred genes and regions associated with a predisposition to autism. An open question is whether different gene mutations will result in different patterns of neural dynamics, or whether different mutations cause a convergent computational phenotype. If multiple gene mutations result in convergent circuit changes, it might provide a common target for the treatment of autism. In order to investigate this, we are developing scalable 3-D neural recording technologies capable of recording single units at thousands of sites throughout the mammalian brain. Using silicon microfabrication techniques, we are implementing high-density 2-D as well as 3-D microelectrode arrays capable of covering large brain volumes, as well as custom electronics and software for scalable data acquisition and automated data analysis. Ultra-dense wiring further allows us to fabricate tightly packed recording sites, to provide the ability for each probe needle to oversample surrounding neurons, and enabling algorithms to aid in spike sorting of the neural data.

Imaging the endogenous Nitric Oxide Signaling and S-nitrosation in neurons

U. I. SENEVIRATNE, G. GONG, D. SCHMIDT, L. C. GODOY, J. S. WISHNOK, G. N. WOGAN, E. BOYDEN, S.R. TANNENBAUM

Presented by: Uthpala I. Seneviratne, Guanyu Gong

Nitric oxide (NO) is produced by three nitric oxide synthase isoforms (NOS1, NOS2 and NOS3) at low levels as signaling molecule and at higher concentrations in pathophysiological conditions. NO? reacts with glutathione (GSH) to form a S-nitrosothiol of glutathione (GSNO) and then is available for transnitrosation to cysteine residues on proteins (RSNOs), as a reversible post-translational modification (PTM) to regulate the activity of enzymes in key biochemical pathways. Until recently, there have been no reliable methods for detecting RSNOs and S-nitrosation as a signaling mechanism. Herein, we present a novel methodology based on phosphine ligation for the detection and absolute quantification of endogenous GSNO in brain tissues. Further, we extended the assay to an imaging technique for S-nitrosation at single cellular level using a phosphine containing cyanine-based fluorophore. Our recent data highlights that in a healthy neonatal mouse different regions of the brain have different amounts of S-

nitrosation, and in a co-culture of neurons and microglial cells, a differential S-nitrosation. Collectively, these findings establish the tools to probe S-nitrosation in the intact nervous system and could illuminate the role of how NO, critical in many other organ systems, plays a major role in the brain.

Effect of emotional distracters on spatial attention in macaque monkeys

R. LANDMAN, J. SHARMA, M. SUR, R. DESIMONE

Presented by: Jitendra Sharma, Rogier Landman

Visual attention involves selection of what is relevant while ignoring what is irrelevant. However, some irrelevant stimuli are harder to ignore than others. In primates, social and emotional stimuli such as faces of conspecifics may attract attention even when 'irrelevant' to the task at hand. The amount of resources directed to those stimuli and whether they attract or repel direct gaze can be measured by the way in which they affect a primary task. Here we varied emotional content in face pictures used as distracters to test their effect on behavioral performance on a spatial attention task in monkeys. In addition we sought to vary monkeys? sensitivity using intranasal administration of the hormone Oxytocin. Two monkeys were trained to perform an attention task in which they monitored one of two gratings for a subtle color change. The monkeys were rewarded for making a saccade to the target grating when it changed. At random times before the target distracter changes (50, 200 or 500ms before the change), two irrelevant images would appear. One of the images could be an image of a monkey face, with a neutral, threatening or a fearful facial expression. The images were located between the fixation spot and the gratings. Reaction times, accuracy and eye movements were analyzed. We find that face images negatively affected accuracy while decreasing reaction time. There was a significant effect of image duration on accuracy with lower accuracy for larger image duration (ANOVA $F(3,16)=75.79$, $p<0.001$). Among trials with faces, reaction time was slower when the face was located near the target grating than when it was located near thedistractersgrating (Kruskal-Wallis test $H(1)=5.26$, $p=0.02$). Accuracy was more strongly affected when the facial expression was fearful or threatening than when the expression was neutral.Intranasal Oxytocin slowed reaction times while improving the accuracy in trials with emotional faces. The data support the notion that emotional distractors compete with learned attentional tasks even when they are irrelevant, most likely because they draw away available attentional resources. Oxytocin reduces these effects in line with the prevalent view that it reduces social fear.

Shank Proteins Regulate Hippocampal Synaptic Transmission

R. SHI, P. REDMAN, Y. LIU, K. JONES, W. XU

Presented by: Rebecca Shi

The postsynaptic density (PSD) of excitatory glutamatergic synapses contains many PDZ-proteins that assemble into a large molecular scaffold, including the Shank (SH3 and multiple ankyrin repeat domains) family. Shank proteins are encoded by three genes (Shank1, Shank2, and Shank3). Shank3 in particular has been implicated in the Phelen-McDermid Syndrome, which is characterized by dysmorphic facial features, delayed expressive speech, and autistic behavior. This project seeks to determine the function of Shanks in mature neurons and examine functional differences between different family members. Shank proteins were knocked down using small hairpin RNAs (shRNAs), and verified with Western blotting. Simultaneous dual whole cell recording was used to measure excitatory postsynaptic currents (EPSCs) at AMPA and NMDA receptors in hippocampal slices. Quantitative Western blotting was also used to examine the developmental profile of Shank protein expression in rat brains. Our results show that

knockdown of different Shanks has differential effects on AMPAR and NMDAR eEPSCs. Combined knockdown of Shank1 and Shank2 shows the greatest effect on eEPSC amplitude, while knockdown of Shank3 seems to have no effect on synaptic transmission. This observation may be explained by relatively low expression of Shank3 at early developmental stages, but invites further investigation into the molecular mechanism by which Shank3 is involved in autism spectrum disorders.

A novel epigenetic mechanism for regulation of synaptic transmission via microRNA-137
S. SIEGERT, E. J. KWON, J. SEO, S. CHO, A. RUDENKO, W. WANG, Z. FLOOD, A. J. MARTORELL, M. ERICSSON, A. E. MUNGENAST, L.-H. TSAI
Presented by: Sandra Siegert
Micro-RNAs are short non-coding RNAs that modulate the expression of hundreds of genes simultaneously and can impact cellular pathways such as for learning and memory. Several single nucleotide polymorphisms (SNPs) in the microRNA-137 gene locus (MIR137) have been associated with autism and schizophrenia and in genome-wide association studies. We examined levels of miR-137 in induced neurons from patients carrying the disease-associated SNP rs2660304, and found that miR-137 is increased in these neurons, compared to controls. We then sought to determine the effect of miR-137 overexpression (OE) in vivo in the mouse brain. Overexpression of miR-137 in the mouse dentate gyrus leads to learning and memory deficits, as well as reduced long- and short-term plasticity at the mossy fiber synapse in hippocampal area CA3. Alterations in the paired-pulse ratio and frequency facilitation at this synapse suggested a likely role for miR-137 in presynaptic transmission. When we examined the mossy fiber pre-synaptic compartment using electron microscopy, we observed marked changes in the distribution of the synaptic vesicle pool in the presence of miR-137 overexpression. To explore the mechanisms by which miR-137 OE may disrupt presynaptic transmission, we examined predicted miR-137 targets that are also known to be presynaptic proteins. MiR-137 directly downregulates the expression of four proteins involved in vesicle trafficking: Complexin-1, N-ethylmaleimide sensitive fusion protein, Synapsin-3, and Synaptotagmin-1. When we restored Synaptotagmin-1 expression in the condition of miR-137 overexpression, we observed a partial rescue of the synaptic plasticity and ultrastructure phenotypes. Our data provide the first mechanistic insight into how the dysregulation of a microRNA can lead to presynaptic defects in neurotransmitter signaling and, as a consequence, leads to cognitive dysfunction in neurodevelopmental disorders such as autism.
Symposium on the Emerging Genetics and Neurobiology of Severe Mental Illness, 2013

Novel genetic technologies for investigating neural circuitry
I. WICKERSHAM
Presented by: Ian Wickersham
Progress in neuroscience is limited by the state of technological development. We have recently begun the 'Genetic Neuroengineering Group' to advance neuroscience by developing and distributing novel tools for genetically manipulating neural circuitry. Current projects range from the relatively modest, such as refinement of monosynaptic tracing tools and development of vectors for long-term expression of multiple transgenes at high levels, to the more ambitious, including development of nontoxic transsynaptic tracers as well as vectors for cell-type-specific transgene expression in wild-type animals.

SnapTag: Tagging Active Neuronal Ensembles Using a Strong Neuronal Activity Promoter (SNAP)

A. YOUNG, K. RAMAMOORTHI, A. SÖRENSON, F.-J. WENG, C. SCHNEIDER, M. BARATTA, R. B. LU, L. BLACHORSKY, Y. LIN

Presented by: Andrew Young

Within neural networks, signals propagated from the external environment are capable of profoundly altering the structure and function of downstream circuitry. Learning via environmental exploration or conditioned stimuli induces functional plasticity in brain circuits including the hippocampus and neocortex. These experiences upregulate genes within active cells. Amongst these activity-regulated genes, several are implicated in the long-term modification of synaptic properties such as ARC, Fos and Npas4. Although these genes act as de facto markers of neuronal activity and concomitant plasticity, their utility in probing plastic changes within active neurons is limited by currently available technology. Using a synthetic biology approach we created a reporter system optimized to selectively target active cells with exquisite temporal fidelity, allowing for unprecedented access to physiological examination and perturbation of active neural circuits. For its construction, we selected a putative Npas4 binding sequence. We based this upon our previous work identifying Npas4?s critical role in memory formation, as well as the observation that it co-localizes with activity regulated transcriptional regulators such as CBP, SRF, and RNA Pol II on several IEGs. We reasoned that a reporter based on an Npas4 binding sequence may act as a robust marker of neuronal activity, integrating activity from several regulatory factors, and granting us access to neuronal populations of interest without restricting focus to a single gene such as Arc or c-Fos.

McGovern Institute Scientific Advisory Board, 2013

Characterization of glial-neuronal signaling pathways disrupted in the Zydeco mutation

Y. V. ZHANG, J. E. MELOM, J. T. LITTLETON

Presented by: Yao Zhang

Precise regulation of neuroexcitability is of critical importance for the function of the nervous system. Impaired excitability in the central nervous system can lead to severe neurological disorders, including epilepsy. In recent years, abnormal glial modulation of neuronal function has emerged as a candidate pathogenic pathway in some epilepsies. Previous work in our lab identified Zydeco (Zyd), a cortex glial sodium-potassium dependent calcium 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. Knockdown of Calmodulin in zyd cortex glia ameliorates the temperature-sensitive seizure phenotype. These observations suggest a calcium-dependent 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. We are particularly interested in identifying how elevated calcium in cortex glia triggers neuronal seizures, and if secretion of a glia-derived neuroexcitative signal may be a culprit. To test this model, we performed an RNAi screen to knock down candidate genes in glia and neurons in the zyd background using repo-Gal4 and elavC155- Gal4, respectively. Suppressors and enhancers of the temperature-sensitive seizure zyd phenotype will be characterized to determine how they

alter glial-neuronal signaling. We will present the progress of these screens as a mechanism to understand how candidate interacting loci regulate acute glial-neuronal communication.
Neurobiology of Drosophila Meeting at CSHL, October 2013

Probing interactions between distinct cortical microcircuits through spatiotemporally patterned 3-D optogenetics

A.N. ZORZOS, T.J. BUSCHMAN, P.E. MONAHAN, J. SCHOLVIN, C. FONSTAD, E.S. BOYDEN

Presented by: Anthony Zorzos

There is a disconnect between experiments done at the level of interactions between brain regions and microcircuit experiments that relate to precise connections between defined cell types. On the one hand, the vast majority of the synapses within a microcircuit come from local neurons; on the other hand, macroscopic measures of neural coordination (e.g., LFP, EEG) reveal evidence for global interactions across many microcircuits distributed throughout the brain. An open question is how neurons within a region balance their responsibility to local vs. distant inputs. To reveal principles of how multiple microcircuits might interact with one another, we implanted a 3-D optogenetic array (Zorzos et al. Optics Letters, 2012) into sensory cortex (SI) of mice expressing channelrhodopsin-2, and recorded single units in motor cortex (MI). By playing back essentially arbitrary '4D' patterns in SI, we observe selective patterns in MI. We observed that cortico-cortical communication is regulated by the same kinds of lateral inhibition (e.g., center/surround receptive field) architectures that govern well-known local circuits in retina and VI; exploring the temporal parameters of how such inhibition is engaged may reveal rules regulating the ability of one part of the brain to control another.
SFN 2013