Grass Reunion Poster Presentations
Noon-1pm, Saturday, July 23; Swope 2nd floor lobby

Jorges Contreras, Rutgers

Mechanism of Gating by Calcium in Connexin Hemichannels

Aberrant opening of non-junctional connexin hemichannels at the plasma membrane is associated with many diseases including ischemia and muscular dystrophy. Proper control of hemichannel opening is essential to maintain cell viability and is achieved by physiological levels of extracellular Ca^{2+}, which drastically reduce hemichannel activity. Here we examined the role of conserved charged residues that form electrostatic networks near the extracellular entrance of the connexin pore, a region thought to be involved in gating rearrangements of hemichannels. Molecular dynamics simulations indicate discrete sites for Ca^{2+} interaction and consequent disruption of salt bridges in the open hemichannels. Experimentally, we found that disruption of these salt bridges by mutations facilitates hemichannel closing. Two negatively charged residues in these networks are putative Ca^{2+} binding sites, forming a Ca^{2+}-gating ring near the extracellular entrance of the pore. Accessibility studies showed that this Ca^{2+}-bound gating ring does not prevent access of ions or small molecules to positions deeper into the pore, indicating that the physical gate is below the Ca^{2+}-gating ring. We conclude that intra- and inter-subunit electrostatic networks at the extracellular entrance of the hemichannel pore play critical roles in hemichannel gating reactions and are tightly controlled by extracellular Ca^{2+}. Our findings provide a general mechanism for Ca^{2+} gating amount different connexin hemichannels isoforms.

Rooma Desai, Mass. General Hospital

Contrasting actions of a convulsant barbiturate and its anticonvulsant enantiomer on the α1 β3 γ2L GABAA receptor account for their i*n vivo* effects

Most barbiturates are anaesthetics but a few unexpectedly are convulsants. We recently located the anaesthetic sites on GABAA receptors (GABAA Rs) by photolabelling with an anaesthetic barbiturate. To apply the same strategy to locate the convulsant sites requires the creation and mechanistic characterization of a suitable agent. We synthesized enantiomers of a novel, photoactivable barbiturate, 1-methyl-5-propyly-5-(m-trifluoromethyldiazirinyl) phenyl barbituric acid (mTFD-MPPB). In mice, S-mTFD-MPPB acted as a convulsant, whereas R-mTFD-MPPB acted as an anticonvulsant. Using patch clamp electrophysiology and fast solution exchange on recombinant human α1 β3 γ2L GABAA Rs expressed in HEK cells, we found that S-mTFD-MPPB inhibited GABA-induced currents, whereas R-mTFD-MPPB enhanced them. S-mTFD-MPPB caused inhibition by binding to either of two inhibitory sites on open channels with bimolecular kinetics. It also inhibited closed, resting state receptors at similar concentrations, decreasing the channel opening rate and shifting the GABA concentration-response curve to the right. R-mTFD-MPPB, like most anaesthetics, enhanced receptor gating by rapidly binding to allosteric sites on open channels, initiating a rate-limiting conformation change to stabilized open channel states. These states had slower closing rates, thus shifting the GABA concentration-response curve to the left. Under conditions when most GABAA Rs were open, an inhibitory action of R-mTFD-MPPB was revealed that had a similar IC50 to that of S-mTFD-MPPB. Thus, the inhibitory sites are not enantioselective, and the convulsant action of S-mTFD-MPPB results from its negligible affinity for the enhancing, anaesthetic sites. Interactions with these two classes of barbiturate binding sites on GABAA Rs underlie the enantiomers' different pharmacological activities in mice.

Scott Fraser, University of Southern California

Structural and functional imaging of live whole brains with light sheet microscopy

Light sheet microscopy, also known as Selective Plane Illumination Microscopy (SPIM), has emerged in recent years as the modality of choice to deliver high, balanced performance in resolution, speed, and penetration depth, while minimizing photo-induced damages (fluorophore bleaching and cellular toxicity). We present our results of utilizing SPIM to image both the structure and function of live, intact, larval zebrafish brains. In one-photon (1p) excitation mode, SPIM delivers the high signal rate and signal-to-noise ratio necessary for us to visualize synapses that have been fluorescently labeled, at endogenous concentration, with recombinant probes. In two-photon (2p) excitation mode, SPIM delivers higher penetration depth than the 1p-counterpart, though with lower signal rate (due to lower 2p absorption cross section), but still is more than 10 times faster than conventional 2p point-scanning microscopy. The near-infrared light of 2p excitation, invisible to most animals' eyes, is critical in allowing observation of brain activity while not perturbing the animal's behavior. We employ 2p-SPIM to map the Calcium-

dependent brain activity of larval zebrafish – the minimal photo-induced damage allows us to continuously record for extended periods of twelve hours or more. Together, the ability to image both the structure, at synaptic resolution, and function, at whole brain coverage, lays the foundation for our ongoing studies to understand the information coding and processing involved in learning and memory, within the context of neuronal homeostasis.

Haleh Fotowat, University of Ottawa
Sensory Evoked Serotonin Dynamics and its Relation to ongoing Communication Behavior

The brain serotonergic (5-hydroxytryptamine; 5-HT) system affects targets throughout the central nervous system and is highly conserved across vertebrates. 5-HT affects the response properties of neurons in sensory cortices of various vertebrate species, but its mechanisms of action under behaviorally relevant conditions remain poorly understood. We used fast-scan cyclic voltammetry in the electrosensory system of weakly electric fish *Apteronotus leptorhynchus* to study sensory-evoked dynamics of 5-HT release in response to naturalistic communication signals. We found that the presence of a conspecific could give rise to 5-HT release specifically in the electrosensory lobe (ELL) map that is specialized for processing electrocommunication signals (lateral segment: LS). The release was variable across fish and in response to conspecifics of different size and sex, suggesting dependence of the response on an individual's past experience. Intense auditory stimuli did not evoke a response, suggesting specificity for the electrosensory modality. Interestingly, 5-HT responses were particularly suppressed in the trials where the fish generated a behavioral response to signals of same-sex conspecifics (chirps). Serotonin release in sensory regions is therefore under the influence of an animal's own behavior and may enhance discrimination of communication signals in a context dependent manner.

Helen Xun Hou, Harvard Medical School
Central Control Circuit for Context-dependent Micturition

Urine release (micturition) serves an essential physiological function as well as a critical role in social communication in many animals. Here we show a combined effect of olfaction and social hierarchy on micturition patterns in adult male mice, confirming the existence of a micturition control center that integrates pro- and anti-micturition cues. Furthermore, we demonstrate that a cluster of neurons expressing corticotropin-releasing hormone (Crh) in the pontine micturition center (PMC) are electrophysiologically distinct from their Crh-negative neighbors and send glutamatergic projections to the spinal cord. The activity of PMC Crh-expressing neurons correlates with and is sufficient to drive bladder contraction, and when silenced impairs micturition behavior. These neurons receive convergent input from widespread higher brain areas that are capable of carrying diverse pro- and anti-micturition signals, and whose activity modulates hierarchy-dependent micturition. Taken together, our results indicate that PMC Crh-expressing neurons are likely the integration center for context-dependent micturition behavior.

David Glanzman, UCLA
Evidence against the synaptic storage of long-term memory in *Aplysia*

We investigated the role of protein synthesis inhibition and DNA methylation in the consolidation of the long-term memory (LTM) for behavioral sensitization in *Aplysia*. Protein synthesis inhibition (PSI) that commenced following long-term sensitization training produced retrograde amnesia, as indicated by the absence of sensitization at 24 h; LTM could be reinstated, however, by truncated sensitization training. By contrast, PSI during long-term training blocked the induction and subsequent consolidation of LTM, as indicated by failure to reinstate memory. Inhibition of DNA methyltransferase (DNMT), whether during or shortly after long-term training, also blocked the consolidation of LTM. Our results point to a central regulative role for protein synthesis-dependent DNA methylation in the consolidation of LTM in *Aplysia*.

George Kracke, University of Missouri School of Medicine
Boron Containing Local Anesthetics Demonstrate Isomer-dependent Analgesia in Mice

Clinically there is a need for local anesthetics with a greater specificity of action and longer duration. We have synthesized a series of local anesthetic derivatives we call boronicaines in which the benzene ring of lidocaine is replaced with ortho-, meta-, dimethyl meta- and para-carborane clusters. A carborane cluster is an icosahedral cage comprised of ten boron and two carbon atoms (C2B10H12) which is more hydrophobic than a benzene ring. The boronicaine derivatives were tested for their analgesic activity and compared to lidocaine using standard procedures

in mice following a plantar injection. The compounds differed in their analgesic activity in the following order: ortho-carborane = dimethyl meta-carborane > para-carborane > lidocaine > meta-carborane derivative. Both ortho-boronicaine and dimethyl meta-boronicaine had longer durations of analgesia than lidocaine. Differences in analgesic efficacies are rationalized by variations in chemical structure and protein binding characteristics. Preliminary studies using voltage gated Na channels expressed in oocytes show that these compounds block channel currents in a state dependent and reversible manner. Financial support to G.R.K. from the University of Missouri Intellectual Property Fast Track Funding Program is acknowledged.

Angelica Lopez, Universidad Nacional Autónoma de México

Defective trafficking in mutant CNG channels

Plasma membrane proteins mediate interaction between cells and the external environment; this makes them excellent therapeutic targets for developing new drugs modulating cell function. Ion channels are membrane proteins that allow ion flow in a regulated manner into or out of the cell by activating signaling cascades in response to different stimuli. Mutations in these proteins are related to some associated diseases called "channelopathies". A simple nucleotide change could induce the inappropriate folding of mutant proteins, leading to premature degradation or intracellular retention. Sometimes the mutant protein is potentially functional but its function is impaired because it cannot reach plasma membrane. In the best scenario the population of mutant proteins reaching the final destination is diminished thereby affecting their function. Cyclic nucleotide dependent ion channels (CNG channels) involved in signal transduction for vision and smell, are related to various channelopathies and represent a good example of membrane proteins that induce cellular localization defects when the protein is mutated. As a first approach towards understanding the mechanisms related to defects in traffic of various mutant CNG channel associated pathologies, we determined the intracellular localization of five CNG channel mutants expressed in HEK-293, analyzing the co-localization using fluorescent intracellular markers by confocal microscopy.

Joaquin Lugo, Baylor University

The Effect of Early-life Status Epilepticus on Ultrasonic Vocalizations in Mice

Infant crying is a series of innate vocal patterns intended to elicit the attention of adult caregivers for fulfillment of specific needs, such as pain, hunger, or hypostimulation. It is one of the earliest forms of observable communication. In neonatal rodents, this behavior has recently been investigated as a potential early behavioral marker of neural deficits in neurodevelopmental disorders. However, few studies have examined the effects of seizures on vocalization behavior during the neonatal period. The purpose of this study is to investigate the effect of a single kainate-induced early life seizure on vocalization behavior in mice. This study also investigates the subsequent effect of seizures on two pathways critical for early neural development and epileptogenesis: the PI3K-Akt-mTOR and Canonical Wnt intracellular signaling pathways.

Reyna Martinez-De Luna, State University of New York Upstate Medical University

Müller Glia reactivity follows retinal injury despite the absence of the Glial Fibrillary Acidic Protein gene in *Xenopus*

Glial fibrillary acidic protein (GFAP) is a class III intermediate filament protein (IFP) that is up-regulated in Müller glia in response to injury or disease, and is thought to play a role in the extensive structural changes observed during Müller cell hypertrophy and glial scar formation. We previously demonstrated that *Xenopus laevis* Müller glia become reactive and hypertrophy following rod loss in an inducible (XOPNTR) model of retinal degeneration. The purpose of this study was to determine if GFAP is required for Müller cell reactivity in *X. laevis*. Methods: Blastn, blastp, and tblastn searches of NCBI and Ensembl databases were used in an attempt to identify a *Xenopus* GFAP ortholog. MAFFT was used to align IFP sequences, identify conserved regions, and generate phylogenetic trees. Retinal injuries included photoreceptor ablation by metronidazole (Mtz) treatment of XOPNTR transgenic tadpoles and retinal ganglion cell (RGC) axotomy. Antibody specificity and changes in IFP expression were determined by western blot, immunohistochemistry and *in situ* hybridization of retinal sections. Degenerate PCR was used to detect gfap genes in amphibian and other vertebrate species. Results: In spite of the morphological changes and GFAP-like immunoreactivity in Müller cells following retinal injury, we discovered that *Xenopus* lack a gene for gfap. Commonly used GFAP antibodies were not specific and also detect the class III IFPs Vim, Prph, Des, and Ina; suggested that one or more of these proteins was upregulated in Müller cells following retinal injury in *Xenopus*. Consistent with this observation, we found that Vim and Prph were significantly induced following

both rod photoreceptor ablation and RGC axotomy. Analyses of the *X. tropicalis* and *X. laevis* genomes indicated either a small deletion or incomplete inversion event resulted in deletion of the gfap gene during evolution. A PCR-based survey of representative species from all three extant amphibian orders (Anura, Caudata and Gymnophiona) suggests deletion of the gfap locus occurred in the ancestor of all Anura after its divergence from the Caudata ancestor around 290 million years ago. Conclusions: Our results demonstrate that extensive changes in Müller cell morphology following retinal injury do not require GFAP in *X. laevis*. Instead, Müller cell changes may require other class III IFPs, potentially including Vim and Prph.

John Meitzen, North Carolina State University

Excitatory synaptic input differs by sex in the nucleus accumbens core but not shell

Sex differences exist in how the brain mediates motivated behavior and reward, both in normal and pathological contexts. Investigations into the underlying neural mechanisms yield accumulating evidence of sexually different dendritic spine morphology and neuromodulator and steroid sex hormone action in the striatal brain regions, including the nucleus accumbens core and shell. How these sex differences influence the electrophysiological properties of neurons in the nucleus accumbens to ultimately modulate this region's function is an area of active research. One current hypothesis is that the excitatory synaptic input onto medium spiny neurons (MSNs), the primary output neurons of the nucleus accumbens, differs by sex. Here we test this hypothesis by performing whole-cell recordings of MSNs in acute brain slices from pre-pubertal male and female nucleus accumbens core and shell. We assess intrinsic neuronal electrophysiological properties through the application of current stimuli in current-clamp and excitatory synaptic input through recording of miniature excitatory post-synaptic currents (mEPSCs) in voltage-clamp. mEPSC frequency is higher in female than in male MSNs in the core but not shell. No sex difference was found in mEPSC amplitude or time of decay. MSN intrinsic excitability and action potential properties are stable across sex in both the core and shell. This data implicates excitatory synaptic input as a potential mechanism underlying sex differences in nucleus accumbens-mediated behaviors, and that sex differences in excitatory synaptic input is generated before puberty.

Joffre Mercier, Brock University

Cell-selective modulation of *Drosophila* muscles by two neuropeptides

Neuropeptides play important roles as modulators of synaptic transmission and neural activity, and they can be released as co-transmitters or as neurohormones. Others have shown that the human nervous system contains over 100 neuropeptides, and a single arthropod species typically expresses a few hundred peptides; over 6,000 distinct prepopeptides are expressed across 10 animal phyla. The occurrence of so many neuropeptides appears to be due at least in part to the ability of distinct peptides to activate selected neurons and inhibit others, leading to the recruitment of selected neurons when neural circuits with specific functions are activated. Our recent work with 3rd instar larvae of *Drosophila* has shown that, in addition to selective modulation of neurons, neuropeptides can act directly on muscle cells to elicit greater modulatory effects on some fibers than others. DPKQDFMRFamide, a *Drosophila* FMRFamide peptide that appears to act as a neurohormone, enhances contractions of muscle cells 6 and 7 more strongly than those of muscle cells 12 and 13. Another peptide, proctolin (RYPLT), enhances contractions more strongly in muscle cells 12 and 13 than 6 and 7. These postsynaptic effects of proctolin correlate with its preferential release as a co-transmitter on muscle cells 12 and 13. Our results demonstrate that cell-selective modulation by neuropeptides is not restricted to neurons and suggest that muscles cells might be selectively targeted by neuropeptides when the neurons controlling them are activated. The functional implications of the complementary effects of proctolin and DPKQDFMRFamide and the mechanisms of action of these two neuropeptides are currently under investigation. Supported by NSERC Canada.

Lisa Moore, Florida Institute of Technology

Catecholamine-Induced Degradation of Vascular Gap Junctions Suggests Mechanism for Stress Cardiomyopathy

Elevated catecholamine levels can cause significant cardiac compromise termed stress cardiomyopathy or Takotsubo syndrome. The clinical setting arises from stress, organophosphates or scorpion toxin. The cause is unknown and is associated with severe left ventricular (LV) dysfunction and malignant arrhythmias. Gap junctions are ion channels that play a role in modulating vascular tone and maintaining cardiac synchronicity. Alteration of the expression of the connexin (Cx) proteins or a change in the ratio of one to the other results in aberrant vascular

and cardiac function.
Vascular smooth muscle cells were treated with epinephrine or its metabolite adrenolutin in a dose and time dependent manner and evaluated by immunoblotting/immunoprecipitation (IP), and immunolabeling with confocal microscopy to assess changes in Cx expression.
Substantial modulation of the gap junction proteins Cx40 and Cx43 occurred with exposure to high dose epinephrine. The loss of Cx43 and Cx40 in the membrane was associated with increased Cx40/43 antibody immunoreactive label in the cytoplasm. This effect was reduced by pre-treatment with SB203580, a p38MAPK (Mitogen activated protein kinase) inhibitor or by propranolol, a non-selective beta-receptor blocker. To address whether the Cx proteins are being targeted for degradation via a ubiquitin-mediated pathway, a mediator of this pathway, epidermal growth factor substrate (EPS15) antibody was used to assess for co-localization with Cx40 and Cx43. Both IP and confocal microscopy results demonstrate that Cx40 and Cx43 are targeted for degradation.
The results indicate sustained catecholamine levels result in a decrease in functional Cx40/Cx43 at the cell membrane. This effect is regulated by increased p38MAPK levels and involves degradation via a ubiquitin-mediated pathway. Although the modulation of Cx43 by p38MAPK and degradation via ubiquitination has been demonstrated, this is the first report indicating stress catecholamine exposure leads to p38MAPK regulation of Cx43/Cx40 and degradation via the ubiquitin pathway. Removal of these important proteins via a degradation pathway may result in cardiac dysfunction such as LV akinesis and atrial and ventricular tachyarrhythmias.

Sandra Rieger, The Jackson Laboratory

Paclitaxel-induced epithelial damage and ectopic MMP-13 expression promotes neurotoxicity in zebrafish

Paclitaxel is a microtubule-stabilizing chemotherapeutic agent that is widely used in the treatment of lung, head and neck, breast and ovarian cancer and in a number of curative and palliative regimens. Despite its beneficial effects, paclitaxel also damages healthy tissues, most prominently the peripheral sensory nervous system. Paclitaxel-induced peripheral neuropathy (PIPN) is common and occurs in ~70-80% of patients with varying severity grades. The most severely affected patients need to terminate chemotherapy, which deprives them of the full benefits of cancer treatment. The mechanisms underlying PIPN remain elusive and therapies that prevent or alleviate this condition are not available. A common view is that paclitaxel-induced microtubule stabilization and transport defects, and increased reactive oxygen species (ROS) formation promote sensory axon degeneration. To date, it remains unclear whether these defects are the cause of axon degeneration or secondary effects. We established a zebrafish *in vivo* model to address this question. Our data showed unexpectedly that not axons but epidermal keratinocytes are a primary target of paclitaxel toxicity. Zebrafish treated with paclitaxel are susceptible to injury by mechanical stress, and show increased expression of MMP-13 (collagenase 3) in keratinocytes following a short treatment period. Axons, in contrast, degenerate at a much later time point and do not display phenotypic changes until the onset of degeneration. Inhibition of MMP-13 activity with two MMP-13 selective inhibitors prevented axon degeneration, suggesting that perturbations in epidermal cell adhesion play a role. We will present new evidence of paclitaxel-dependent MMP-13 regulation and function.

Adrian Rodriguez-Contreras, City College of New York

The Effect of whisker plucking on the interaction between astrocytes and blood vessels in the barrel cortex of juvenile mice

Astrocytes interact with arteries and microcapillaries through end feet connections that are thought to be involved in neurovascular coupling and the maintenance of the blood brain barrier. Recent studies indicate that manipulations of sensory input affect the development of the vascular network (Lacoste *et al*., 2014; Whitheus *et al*., 2014). We hypothesize that depriving sensory input will result in a decreased interaction between astrocytes and blood vessels. To test this hypothesis, we examined the mouse barrel cortex, a model system for studying sensory-dependent plasticity during postnatal development. We used a unilateral whisker-plucking paradigm to reduce activity in the barrel cortex of juvenile mice expressing Cre recombinase driven by the GFAP promoter. The left set of whiskers were plucked daily from postnatal day 15 (P15) until P21, and the right set of whiskers were left intact and used as the control (n=3 mice). At P21, the mice were perfused with fixative solution and their brains were processed with multiple fluorescence labeling using IB4 histochemistry to label blood vessels and anti-GFAP or anti-ALdh1L1 immunohistochemistry to label astrocytes (2-4 slices per mouse). We used confocal imaging and 3D segmentation analysis to visualize and measure vessel and astrocyte labeling volume, and astrocyte-vessel interactions in cortical layer 4 of the barrel cortex. Our preliminary results show that IB4 volume in controls (mean

± sem, in μm3) was 12526 ± 324 in double-labeled ALdh1L1/IB4 slices and 8532 ± 942 in double-labeled GFAP/IB4 slices. IB4 volume in the whisker pluck condition (mean ± sem, in μm3) was 12157 ± 344 in double-labeled ALdh1L1/IB4 slices and 8115 ± 990 in double-labeled GFAP/IB4 slices. This is an approximate 3-5% decrease in blood vessel volume in whisker pluck compared to control. However, we did not find apparent changes in astrocyte marker volume. Our next step is to determine if whisker plucking affects astrocyte-vessel interactions during the critical period of vascular development. In current experiments, we are using the same unilateral whisker plucking paradigm, with the difference of whisker-plucking taking place within P0 - P5, the critical period for sensory development and vascular growth in the mouse barrel cortex.

Joshua Salvi, The Rockefeller University

Control of a sensory hair bundle's function by its mechanical load

Sensory hair bundles in the auditory system detect sound and those in the vestibular system detect head position and rotation. As a bundle's function changes, so too do its mechanical properties. Hair bundles range in height from 1 μm to 100 μm and in stiffness from 0.1 mN•m 1 to 10 mN•m 1. They possess stereocilia that vary in number from fewer than 20 to more than 300. Hair bundles may be free-standing or they may be coupled to a tectorial membrane, otolithic membrane, sallet, or cupula. In the auditory system, the bundles are coupled to a tectorial membrane, which possesses a tonotopic gradient stiffness from 10 mN•m 1 to 300 mN•m 1 and a mass gradient from 35 ng to 100 ng per row of hair cells. Vestibular hair bundles of the sacculus and utriculus are instead be coupled to an otolithic membrane with a stiffness of only 1 mN•m 1 and are additionally loaded with an otoconial mass of about 2,000 ng per hair cell. In view of these variations, we hypothesized that a hair bundle's mechanical load controls its sensory function.

We designed a mechanical-load clamp to control for an individual hair bundle both the load stiffness and the constant force, parameters that have previously been predicted to control a bundle's mechanosensory function. The load clamp permitted us to generate a map of hair-bundle behavior—a state diagram—for different combinations of these mechanical loads. Within an ovoid region of the diagram, the bundle oscillated spontaneously, whereas it remained quiescent outside of this regime. The border of the spontaneously oscillatory regime constituted a line of Hopf bifurcations. We delivered periodic mechanical stimuli to a hair bundle situated within different locales of its state diagram and found that the bundle achieved maximal sensitivity, sharpest frequency selectivity, and greatest degree of entrainment when poised close to a Hopf bifurcation. Furthermore, a hair bundle subjected to certain mechanical loads could exhibit a rapid twitch at the onset of a force pulse, a behavior that might be useful in a vestibular organ. Finally, changing the load applied to a frog's vestibular hair bundle, caused it to overshoot its stimulus like a mammalian auditory hair bundle. Upon adjustment of only two mechanical parameters, a single bundle can exhibit a variety of mechanosensory behaviors, implying an essential similarity between the bundles from different organs and organisms.

Honi Sanders, Brandeis University

Hippocampal Theta: Signature of a Repetitive Multi-Part Process

Place cells in the hippocampus preferentially fire when an animal is in a particular location in a given environment. In addition, the firing rate of place cells can change by an order of magnitude in response to non-spatial (e.g. sensory or task-related) information. Not only is information conveyed in the firing rate of place cells (rate remapping), the precise timing of spikes with respect to the ongoing theta cycle (phase coding) has been hypothesized to provide a substrate for linking ordered events.

In this project, we have analyzed in vivo extracellular recordings of place cell spiking activity and the local field potential in dorsal CA1 of rats. The rats were trained on alternation task on a figure 8-shaped maze. Many cells with place fields on the central arm rate remapped depending on which arm the rat is coming from, as previously reported (Ji and Wilson, 2008). We found that rate remapping preferentially occurs during the first half of theta cycles, whereas phase precession preferentially occurs during the second half of theta cycles.

Annalisa Scimemi, SUNY Albany

Glutamate uptake and astrocyte morphology regulation by protease activated receptors

The G-protein coupled, protease-activated receptor 1 (PAR1) is a membrane protein expressed in astrocytic processes in contact with neurons and blood vessels. PAR1 is proteolytically-activated by serine proteases also involved in the formation of blood clots. PAR1 plays a role in thrombosis, hemostasis and inflammation, but whether it also regulates glutamate uptake, a major function of astrocytes, is unknown. Here we show that, in the

mouse hippocampus, PAR1 activation speeds glutamate clearance from astrocytes and induces structural re-organization of astrocytic processes surrounding glutamatergic synapses. Reaction-diffusion simulations indicate that these structural changes can account for the faster rate of astrocytic glutamate uptake induced by PAR1 activation. Together, these findings identify PAR1 as an important regulator of excitatory synaptic transmission in the brain.

Annalisa Scimemi, SUNY Albany

Neuronal glutamate transporters control dopaminergic signaling in the striatum

Glutamate transporters shape the lifetime of glutamate in the extracellular space and regulate the strength of excitatory synaptic transmission in the brain. EAAC1 is a neuronal glutamate transporter abundantly expressed in the striatum. Recent studies suggest that mutations in the gene encoding EAAC1 are associated with the onset of obsessive compulsive disorder, but the molecular mechanisms underlying this effect are not known. Here we show that loss of function of EAAC1 leads to increased anxiety and motor activity in mice. These effects are associated with increased activation of metabotropic glutamate receptors in striatal neurons, altered phosphorylation state of the dopamine and cyclic-AMP regulated phospho-protein DARPP-32, and to changes in the expression of D1 dopamine receptors. These findings may provide a candidate molecular mechanism through which glutamate receptor activation via EAAC1 regulates behaviors that rely on the functional activity of striatal circuits.

Ava Udvadia, University of Wisconsin-Milwaukee

Gene regulatory networks driving successful CNS regeneration

CNS injury in adult mammals fails to stimulate a regenerative response and usually results permanent disability. In contrast, CNS injury in fish elicits a robust regenerative response often leading to almost full recovery of function. Since the basic machinery regulating the wiring of the nervous system is well conserved across vertebrate species, the underlying difference stems from the relative abilities to restart the axon growth machinery in response to CNS injury. Our work is focused on discovering the gene regulatory network underlying successful CNS regeneration in zebrafish. We have discovered a combination of three transcription factors from the bZIP and bHLH transcription factor families that are essential for regeneration-associated expression of GAP-43 and successful target reinnervation in a zebrafish optic nerve transection injury model. We are now investing the mechanisms underlying the synergistic activities of this critical combination.

Fernando Vonhoff, Yale University

Live imaging of activity-dependent synaptic refinement in *Drosophila* embryos

The pruning of off-target contacts is a crucial mechanism for establishing precise neural networks during development. Neural activity plays a key role in synaptic plasticity and refinement in various systems including the vertebrate visual system, where low frequency (0.01 Hz) calcium (Ca) oscillations refine early topographic maps. At the *Drosophila* neuromuscular junction (NMJ) synaptic refinement also occurs in an activity-dependent manner, where oscillatory neural activity and presynaptic Ca signaling modulate the motoneuron's response to the retrograde chemorepellent Sema2a for the removal of off-target contacts. We have analyzed the role of Ca oscillations in embryonic growth cones in vivo using the Ca reporter GCaMP. We used mutations of the myosin heavy chain (MHC) gene to suppress muscle contractions, allowing for time-lapse imaging throughout embryonic development. MHC embryos show normal muscle development, innervation, and motor system maturation, albeit with fictive motor activity. We find that Ca oscillations are observed in both native and ectopic contacts. We are now examining the dynamics of extension and retraction of motoneuron filopodia on correct and incorrect synaptic targets in intact embryos. We have identified several molecular players acting downstream of presynaptic Ca, such as the Ca-dependent adenylyl cyclase Rutabaga, Calcineurin and CaMKII. We optogenetically manipulated cAMP levels by using the photoactivatable adenylyl cyclase bPAC, and showed that presynaptic cAMP levels are sufficient but not necessary to oscillate at a similar frequency than Ca signals for synaptic refinement. We propose that the protein kinase A (PKA) and the protein phosphatase 1 (PP1) act downstream of cAMP to regulate CaMKII function to modulate Sema2a-dependent chemorepulsion acting through its presynaptic receptor PlexinB. These results introduce a model for in vivo analysis of oscillatory second messenger signals in synaptic refinement during NMJ formation.