Abstract #1

EAAT1 P290R mutation reduced glial infiltration in neuropil through disruption of chloride homeostasis

Astrocytes regulate extracellular glutamate levels in the CNS by expressing Excitatory Amino Acid Transporters (EAATs). Mutations of EAAT1 are found in patients with type 6 episodic ataxia (EA6), who may also suffer seizures, migraine, cerebellar atrophy and hemiplegia. One mutation is a P>R substitution (EAAT1 P290R). Overexpression of this mutation reduces endogenous EAAT1 protein levels and glutamate transport, suggesting it acts in dominant-negative fashion. However, it also causes abnormal outward flow of anions, raising the possibility of neomorphic effects. Here we used Drosophila to examine the pathological effects of this mutation on glial cell function in vivo.

Null mutants for *Eaat1* have no defects in glial cell number or neuropil infiltration. However, null larvae have severe locomotor defects that can be rescued by glial expression of wild-type (WT) fly Eaat1 or human EAAT1 (SLC1A3), demonstrating conserved EAAT function. However, the P>R mutations of these proteins did not rescue, indicating their dysfunction in vivo. We overexpressed WT or the P>R mutation in glia, and assessed their effects on astrocyte morphology and larval locomotion. In contrast to WT, overexpression of the P>R mutation led to reduced infiltration of astrocytic processes into the neuropil and reduced locomotion. At later stages, there was also glial cell loss and premature death of the animals. The defects caused by the P>R mutation were clearly distinct from those observed in *Eaat1* null mutants, indicating a neomorphic effect that cannot be explained by loss of glutamate transporter function. We hypothesized that an abnormal outward flow of chloride from astrocytes might cause this neomorphic effect, and tested this with genetic approaches expected to manipulate chloride transport. Misexpression of the K-Cl cotransporter Kcc (which normally pumps chloride out of cells) mimicked the cellular pathology in astrocytes caused by P>R mutation. Remarkably, overexpression in astrocytes of the Na-K-Cl cotransporter Ncc69 (which normally pumps chloride into cells) rescued the effects of the P>R mutation on both cellular pathology and locomotor behavior. Thus dysfunction caused by the P>R mutation likely arises in part from abnormal outward flow of chloride from glia, which may act alone or in combination with reduced glutamate recovery to cause episodic ataxia.

Abstract #2

Drosophila astrocytes and its function at the synapse

Glutamate is the principal excitatory neurotransmitter in the brain, and tight control of extracellular glutamate levels is crucial to avoid over-excitation, toxicity and neuronal cell death. Most extracellular glutamate is safely moved into astrocytes by high affinity excitatory amino acid transporters (EAATs). Expression of EAATs is dysregulated in amyotrophic lateral sclerosis, stroke, epilepsy, schizophrenia, Alzheimer's and Huntington's diseases among others. EAAT mutant mice exhibit spontaneous seizures, and humans with mutations in the EAAT transporter known as
SLC1A3 suffer seizures in addition to episodic ataxia and hemiplegic migraine. Despite this importance for CNS pathologies, the mechanisms of EAAT regulation and pathogenesis are poorly understood. The fruit fly Drosophila is an advanced genetic model with a single high-affinity glutamate transporter Eaat1. Previous work in the our lab has shown that Eaat1 mutant larvae have severe defects of locomotion caused by failure of glial cells to influence glutamatergic neurotransmission controlling the rhythmic patterning of motor neuron activity. We rescued these defects with expression of human SLC1A3, specifically in CNS glia. This demonstrates conserved EAAT function and offers the promise that discoveries in flies can be translated to humans.
To identify factors that regulate Eaat1 and influence over-excitement by glutamate in vivo, we performed an RNA interference (RNAi) screen. Surprisingly, a majority of our positive genes (24) from this screen include receptors, transporters and metabolic enzymes for the neurotransmitters glutamate, GABA, acetylcholine, serotonin and dopamine. This suggests Drosophila glial cells, like glial cells in mammals, respond to neurotransmission. Furthermore, it appears that disruption in these responses can affect 1) alteration in Eaat1 expression, 2) Shape changes in astrocytes, 3) altered development and maintenance of astrocytes and 4) All of the above.
We hypothesize that astrocytes detect multiple neurotransmitters, leading to changes in cell shape and Eaat1 expression/localization that affect glutamatergic neurotransmission in the CNS. Using Gal4-UAS driven membrane-tagged GFP, we are presently identifying how receptors for specific neurotransmitters influence the dynamic morphologies of astrocytes. Astrocytes respond to neuronal activity with changes in calcium dynamics in localized microdomains. Using the genetically encoded calcium indicator (GCamP5), we will measure calcium dynamics in Drosophila astrocytes, and characterize changes upon loss of neurotransmitter receptors with RNAi. We will further test how loss of the ability of astrocytes to detect neurotransmitters can influence integration of neural activity and affect locomotor behavior in larvae. My project will demonstrate how neurotransmitters act on astrocytes to influence structural plasticity, calcium dynamics and behavior.

Abstract #3

Genome-wide DNA methylation in the striatum of individuals with cocaine dependence.

Background: Multiple genetic and environmental factors interact to determine the risk and trajectory of cocaine dependence, and the expression of addiction-associated genes is likely influenced by environmental factors that leave persistent epigenetic marks on an individual's genome. Recently, several studies have identified epigenetic mechanisms that are associated with long term transcriptional and synaptic changes and with the acquisition of compulsive drug seeking in animal models, but little is known about the role of epigenetics in human cocaine dependence. Of particular interest is DNA methylation as it represents a mitotically stable mark that has been shown to be altered by environmental experience. This study aims to identify genome-wide changes in DNA methylation in two cocaine-relevant regions of the human brain, the dorsal and ventral striatum.

Methods: We used Reduced Representation Bisulfite Sequencing (RRBS) on nucleus accumbens and caudate tissue from 25 dependent cocaine users and 25 drug-free and age-matched controls. RRBS is a high throughput sequencing based approach, which enriches for CpG dinucleotides and generates methylation data at base-pair resolution. We will also compare these data to transcriptome-wide RNA sequencing data and deep bisulfite sequencing data in neuronal and non-neuronal nuclei, separated using fluorescently activated cell sorting (FACS).

Results: All RRBS libraries contained more than 4 million reads at 10X coverage and over 65% of reads in each library were uniquely aligned to the human genome. After filtering each library for quality and correcting for covariates, we've identified multiple differentially methylated CpG clusters between groups, in both the ventral and dorsal striatum. In addition, we've uncovered regions of hyper- and hypomethylation that are associated with transcriptional dysregulation, in cases versus controls, for follow-up in sorted nuclear fractions.

Abstract #4

14-3-3 Zeta Regulates Netrin-1 Responses in Spinal Cord Commissural Neurons

During the development, neurons must project their axons towards the right targets in order to establish physiologically relevant connections. In their way, they must interpret a complex molecular environment, a role that is fulfilled by the growth cones. Our groups has previously identified 14-3-3s, a family of adaptor proteins, as being a major constituent of the growth cones, and their importance for axon guidance has been shown in both *in vivo* and *in vitro* models. In the present work, we used Dunn chamber turning assays to show that R18, a 14-3-3 antagonist, abolishes netrin-dependent attraction and fast growth cone responses in pre-crossing spinal cord commissural neurons. In a screen for 14-3-3 isoforms that interact with DCC, we found 14-3-3 zeta as being a major binding partner of this receptor. Interestingly, 14-3-3 zeta knockdown mimicked R18 treatment in our phenotypical assays *in vitro*. Analysis of 14-3-3-null mouse spinal cords revealed that 14-3-3 zeta deficiency causes defects commissural projections. Additional experiments are being performed, in order to analyze the effects of 14-3-3 in DCC signaling.

Abstract #5

Quantification of Protein Levels in Single Cells In Vivo

Accurate measurement of the amount of specific protein a cell produces is important for investigating basic molecular processes. We are developing a technique that allows for quantitation of protein levels in single cells in vivo. This Protein Quantitation Ratioing (PQR) technique uses a genetic tag that produces a stoichiometric ratio of a fluorescent protein reporter and the protein of interest during protein translation. The fluorescence intensity is proportional to the amount of the protein of interest produced and is used to determine the relative protein concentration within the cell. We use PQR to quantify protein expression of different genes using quantitative imaging, electrophysiology, and phenotype. We use genome editing to insert Protein Quantitation Reporters into endogenous genomic loci in three different genomes for quantitation of endogenous protein levels. The PQR technique will allow for a wide range of quantitative experiments examining gene to phenotype relationships with greater accuracy and cellular resolution.

Abstract #6

The Role of Constitutively Active Kinases in Memory Maintenance

Memories are believed to be maintained in the brain through an active enzymatic process, one that is able to prevent decay of synaptic strength and allow the molecular memory trace to persist for long periods of time. A promising candidate for this process of memory maintenance is protein kinase M zeta (PKMζ), the constitutively active form of the atypical PKCζ. The increased synaptic strength observed after activity-dependent long term potentiation (LTP)—one of the most well characterized cellular models for memory—is caused by increased localization of AMPA receptors to the potentiated synapse, a process whose reversal is thought to be inhibited through a PKMζ-mediated pathway. Using the marine mollusc Aplysia californica, we investigate the endocytic protein NUMB as a possible substrate through which PKMz prevents AMPA receptor endocytosis following LTP. We show that PKMz can phosphorylate NUMB in cell culture, and through utilization of a pH-sensitive GFP (pHluorin)-AMPA receptor construct, we hope to show the effects of NUMB phosphorylation on AMPA receptor endocytosis at activated synapses. The molecular mechanisms underlying memory is one of the fundamental mysteries remaining in the field of neuroscience today, and understanding the players involved in this complex process will provide much needed insight into pathological conditions where these processes go awry.

Abstract #7

Heme Oxygenase-1 Modulates microRNA Expression in Cultured Astroglia: Implications for Chronic Brain Disorders

Background: Over-expression of the heme-degrading enzyme, heme oxygenase-1 (HO-1), promotes iron deposition, mitochondrial damage and autophagy in astrocytes and enhances the vulnerability of nearby neuronal constituents to oxidative injury. These neuropathological features and aberrant brain microRNA (miRNA) expression patterns have been implicated in the etiopathogeneses of various neurodevelopmental, such as Schizophrenia, and aging-related neurodegenerative disorders, including Parkinson's disease.
Objective: To correlate glial HO-1 over-expression with altered miRNA patterns, which have been linked to the aforementioned "core" neuropathological features.
Methods: miRNA microchip assays were performed on *HMOX1*- and sham-transfected primary rat astroglia and affected miRNAs were further validated by qPCR. The roles of the heme degradation products, carbon monoxide (CO), iron (Fe) and bilirubin, on miRNA expression were assessed and salient mRNA targets of the impacted miRNAs were ascertained.
Results: In *HMOX1*-transfected astrocytes, rno-miR-140*, rno-miR-17, rno-miR-16 were significantly up-regulated, and rno-miR-297, rno-miR-206, rno-miR-187, rno-miR-181a, rno-miR-29c were down-regulated, compared to sham-transfected controls. CO and Fe were implicated in the *HMOX1* effects, whereas bilirubin was inert or counteracted the *HMOX1*-related changes. mRNA levels of *Ngfr*, *Vglut1*, *Mapk3*, *Tnf-α*, and *Sirt1*, known targets of the down-regulated miRNAs and abnormal in various human brain disorders, were significantly increased in the *HMOX1*-transfected astrocytes.
Conclusions: In chronic CNS disorders, altered expression of salient miRNAs and their mRNA targets may contribute to the neural damage accruing from the over-expression of glial HO-1.

Abstract #8

AMIGO-1 controls axonal targeting the mouse olfactory system

The brain consists of billions of neurons which connect to one another to form immensely complex circuits in the brain. Sensory systems provide a good example of the precise organization that occurs within these circuits in the form of topographic maps. During development, molecular guidance cues as well as neuronal activity together guide the growth of axons and instruct accurate target selection. The mouse olfactory system provides a useful model to study the mechanisms by which primary olfactory sensory neurons find their target neurons in the olfactory bulb. While several molecules have been implicated in the global targeting of olfactory sensory neurons to the olfactory bulb, less is known about the more precise targeting of these sensory neurons onto specific glomeruli in the bulb. The few cell adhesion molecules that have been suggested to play a role in the local targeting of olfactory axons are not sufficient to explain the organization of the huge number of connections that need to be formed. We have identified AMIGO-1 as an additional cell adhesion molecule that contributes to precise olfactory map formation. Findings in the literature based on *in vitro* studies suggest roles for this protein in various developmental functions such as neurite outgrowth and axon fasciculation. Based on these data as well as the expression of AMIGO-1 in the olfactory epithelium, we hypothesized AMIGO-1 to be necessary for targeting in the development of the mouse olfactory system. *Amigo1* knockout mice show defects in the targeting of ventrally located olfactory sensory neurons. Glomeruli innervated by a subset of ventral sensory neurons are smaller, and we find instances of ectopic glomerular innervations. These findings suggest a role for Amigo1 in olfactory map formation. We continue to investigate the mechanisms by which Amigo1 regulates olfactory system development.

Abstract #9

LGI1 regulates central synapse number through an NgR1-RhoA signalling pathway

During central nervous system development, supernumerary synapses are eliminated and in the hippocampus, a complex of Troy and Nogo66 Receptor (NgR1) limits synapse number through a RhoA-dependent mechanism. We have previously identified Leucine-Rich Glioma Inactivated 1 (LGI1) as a novel NgR1 ligand that blocks Nogo66-induced NgR1 signalling, prevents myelin-induced growth cone collapse, and promotes neurite outgrowth on myelin substrates. Here, we test the hypothesis that LGI1 functions to suppress NgR1-dependent synapse pruning in the developing hippocampus.

Consistent with this hypothesis, we found that synaptic density is significantly reduced in $LGI1^{-/-}$ hippocampal neurons examined at 15 or 18 DIV whereas synapse number was increased in $NgR1^{-/-}$ hippocampal neurons. We then determined whether LGI1 deletion resulted in structural changes *in vivo* by quantifying numbers of mushroom, stubby or thin spines present within dendrites of CA1 pyramidal neurons in $LGI1^{-/-}$ hippocampal slices and found that the proportion of mushroom type spines is significantly decreased in $LGI1^{-/-}$ mice versus wild-type littermate controls. To confirm these findings using a functional approach, we assessed mEPSCs generated by CA1 hippocampal neurons within LGI1-/- hippocampal slices and found an increase in mEPSC inter-event interval in $LGI1^{-/-}$ slices. Therefore, LGI1 deletion reduces hippocampal synapse number and function *in vitro* and *in vivo*.

To determine if LGI1 can alter signalling mediated by an NgR1-TROY complex, we employed our recently described cell-based bioassay that provides a read-out for small GTPase activation by p75NTR and related receptors. Using this, we demonstrated that co-expression of NgR1 and TROY induced RhoA-dependent cytoskeletal changes that were blocked when LGI1 was present. This suggested that RhoA activity levels may be higher in primary neurons lacking LGI1 and, consistent with this, we found that levels of active RhoA were increased $LGI1^{-/-}$ neurons compared to their wild-type counterparts. Taken together, these findings indicate that LGI1 regulates synapse number by antagonizing an NgR1 signalling complex that mediates RhoA activation.

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