Abstracts

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GLUCAGON-LIKE PEPTIDE-1 RECEPTOR ACTIVATION IN THE VENTRAL TEGMENTAL AREA OR THE NUCLEUS ACCUMBENS ATTENUATES COCAINE SEEKING IN RATS

Nicole Hernandez

Glucagon-like peptide-1 (GLP-1) receptor signaling in the CNS is pharmacologically and physiologically relevant for energy balance control. The GLP-1 receptor agonist exendin-4 decreases intake of palatable food when administered into the ventral tegmental area (VTA) and nucleus accumbens (NAc) core. Since the VTA and the NAc mediate the reinforcing effects of food and drugs of abuse, we hypothesized that GLP-1 receptor activation in these two nuclei would attenuate cocaine reinstatement, an animal model of relapse in human addicts. Initially, rats were allowed to self-administer cocaine (0.25 mg/infusion i.v.) for 21 days on a fixed-ratio 5 (FR5) schedule of reinforcement. Cocaine self-administration was then extinguished by replacing cocaine with saline. Once cocaine taking was extinguished, rats received an acute priming injection of cocaine (10 mg/kg, i.p.) to reinstate cocaine-seeking behavior. During subsequent reinstatement test sessions, rats were pretreated with intra-cranial infusions of the GLP-1 receptor agonist exendin-4 (0, 0.005 and 0.05 μg) prior to a priming injection of cocaine. Here, we show that administration of exendin-4 directly into the VTA, NAc core or NAc shell dose-dependently attenuated cocaine priming-induced reinstatement of drug-seeking behavior. To determine if the suppressive effects of exendin-4 in the VTA and NAc on cocaine seeking were due to drug-induced motor impairments, we also examined the effects of intra-cranial exendin-4 infusions on the reinstatement of sucrose seeking. Administration of exendin-4 directly into the VTA, NAc core or NAc shell had no effect on sucrose reinstatement. Taken together, these results indicate that increased activation of VTA and NAc GLP-1 receptors is sufficient to reduce cocaine seeking and that these effects are not due to general motor suppressant effects of drug treatment. Thus, these findings support re-purposing GLP-1 receptor agonists, which are FDA-approved for treating diabetes type II and obesity, for treating cocaine addiction.

INVESTIGATING THE ROLE OF THE DENTATE GYRUS IN TEMPORAL LOBE EPILEPSY COGNITIVE COMORBIDITIES

Julia Kahn

Up to 30% of patients diagnosed with epilepsy display intractable symptoms despite treatment, and temporal lobe epilepsy (TLE) is the most prevalent form of the intractable epilepsies. Although epilepsy management focuses on ameliorating seizure activity, patients must cope with a host of complicated comorbidities, which many patients find more detrimental to daily life than the seizures. However, the neural mechanisms mediating these cognitive impairments in epilepsy are not well defined. The dentate gyrus (DG) is fundamental for cognitive functions in the hippocampus, a structure that TLE seizures highly activate. The DG's principal cells, dentate granule cells (DGCs), have been implicated in critical features of episodic memory, particularly pattern separation. Pattern separation is mediated by remarkably sparse activation in the DGCs. Sparse DGC activation has a secondary effect: the DG limits cortical input to the hippocampus and acts like a "gate," the failure of which may contribute to the excessive cortical-hippocampal activity underlying TLE seizures. Our laboratory previously found pronounced changes in DGC activation patterns following epilepsy onset: only 5% of DGCs fire at any given time in a healthy brain, but more than 50% of DGCs activate in our TLE animal model. In this study, we used Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to hyperactivate DGCs in otherwise normal mice to isolate the potential cognitive consequences on a hippocampal memory task. In the spatial object recognition (SOR) task, mice are exposed to 3 objects; 24 hours later, one of the objects is moved, and the amount of time the mice explore the displaced object (DO) reflects if the mice recognize a change in their spatial environment. Wild type mice performed well on the SOR task, spending significantly more time exploring the DO compared to the non-displaced objects (NDOs). However, epileptic mice failed to discriminate between the objects. Mice that received viral injections of the excitatory DREADD (AAV5.CaMKIIa.hM3D.IRES.mCitrine) to the dorsal DG were given either saline or CNO injections i.p. 1 hour before the SOR testing trial. Mice receiving saline successfully discriminated between the DO and NDOs, while the mice that received CNO failed to discriminate. Mice that received DREADDs were a transgenic mouse line that uses the immediate early gene fos to drive expression of tdTomato in active cells when the drug tamoxifen is present, called Fos Targeted Recombinase in Active Populations (FosTRAP). FosTRAP slices provided a snapshot view of DGCs recruited during behavior and by the DREADDs. These data suggest that DREADD-driven hyperactivation of the DGCs was sufficient to compromise behavioral performance. Thus, DGC hyperactivation may be a critical factor in TLE cognitive comorbidities.

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W. Timothy O’Brien

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ASTROCYTES REGULATE GLP-1 RECEPTOR-MEDIATED EFFECTS ON ENERGY BALANCE

David Reiner

The anorectic effects of glucagon-like peptide-1 receptor (GLP-1R) agonists are partly due to direct GLP-1R signaling in the CNS. A small body of literature suggests that GLP-1Rs are expressed on CNS astrocytes. As astrocytes play a critical role in modulating extracellular glutamate, and the hypophagic effects of GLP-1R activation are partially mediated via glutamatergic signaling, GLP-1R agonists may act directly on astrocytes in feeding-relevant nuclei to regulate energy balance. We tested the hypothesis that GLP-1R ligands act on astrocytes within the nucleus tractus solitarius (NTS), a hindbrain nucleus critical for energy balance control, to affect feeding and body weight in rats. Central or peripheral administration of a fluorophore-labeled GLP-1R agonist, exendin-4 (Ex-4), localizes within astrocytes and neurons in the NTS. Live cell calcium imaging of hindbrain slices revealed prolonged activation by Ex-4 of both NTS-astrocytes and neurons (~40%). Application of GLP-1R agonists increases cAMP in immortalized astrocytes. IHC data show that endogenous GLP-1 axons form close synaptic apposition with NTS astrocytes. Pharmacological inhibition of NTS astrocytes with the astrocyte inhibitor fluorocitrate (50mM) significantly attenuates the anorectic and body weight-suppressive effects of intra-NTS Ex-4 (0.05μg). Collectively, data demonstrate a role for NTS astrocytic GLP-1R signaling in energy balance control.

THE IMPACT OF RECOVERY SLEEP OPPORTUNITY ON NEUROBEHAVIORAL MEASURES FOLLOWING CHRONIC SLEEP RESTRICTION

Olga Tkachenko

Introduction: Using a large cohort of experimentally sleep-restricted healthy adults, we examined whether recovery from chronic partial sleep restriction (SR) differed among neurobehavioral measures given varying doses of recovery sleep opportunity. Methods: N=306 adults (21-50y, 46% female) had 2 baseline laboratory sleeps (BL1-2; 10h TIB), then randomization to either a control condition (10h TIB on all nights; n=28) or to 5 SR nights (SR1-5; 4h TIB) followed by randomization to 1 of 7 single-night recovery sleep opportunity conditions (R1; 0, 2, 4, 6, 8, 10, or 12h TIB; n=278). Performance outcomes included the Psychomotor Vigilance Test (PVT), the Digit Symbol Substitution Task (DSST), and subjective outcomes included the Karolinska Sleepiness Scale (KSS) and the fatigue subscale of the Profile of Mood States (POMS-F). Sleep physiology was recorded. Mixed model repeated measures analyses were used to compare changes in outcomes from baseline to post-recovery sleep dose (R1-BL2) between the sleep-restricted and control cohorts. Results: After recovery sleep of less than 6h, SR subjects still differed statistically from controls on all outcomes as measured by change from baseline to post-recovery sleep. With 6h TIB, SR subjects no longer differed from controls on the DSST. After 8h TIB, SR subjects did not differ from controls on the KSS and POMS-F. On the PVT, only subjects given 10 or 12h TIB on the recovery night were able to match performance with the control group. Conclusion: There appears to be a premature perception of full recovery from sleep restriction evident in subjective ratings and cognitive throughput measures, despite continued deficits of attention. Furthermore, distinct components of neurobehavioral functioning require recovery sleep opportunities of different lengths in order to return performance to expected levels.

EFFECT OF COGNITIVE BEHAVIOR THERAPY ON RESTING-STATE AND TASK-EVOKED BRAIN ACTIVITY IN MDD AND PTSD

Zhen Yang

Effect of Cognitive Behavior Therapy on Resting-state and Task-evoked Brain Activity in MDD and PTSD Zhen Yang1, Desmond Oathes1, Stephen E. Bruce2, Theodore D. Satterwatthe1,3, Philip A. Cook1,4, Eli Mikkelsen1, Emma Satchell 1, Russell T. Shinohara1,5, Haochang Shou 1,5, Yvette I. Sheline1,4,6 1Center for Neuromodulation in Depression and Stress, Department of Psychiatry, University of Pennsylvania, Philadelphia, PA19104; 2Center for Trauma Recovery, University of Missouri, St. Louis, MO 63121; Department of Psychiatry; 4Department of Radiology, 5Department of Biostatistics and Epidemiology; 6Department of Neurology, University of Pennsylvania, Philadelphia, PA19104 Introduction: Traditionally, MDD and PTSD were considered separate disorders with distinctive neuropsychopathology. With the advent of the Research Domain Criteria, there has been an emphasis on dimensions of psychopathology that extend across clinical diagnostic criteria to abnormalities in certain brain circuitry. Cognitive behavioral therapy (CBT) is an effective treatment for both MDD and PTSD. Here, we applied a Linear Mixed-Effects (LME) modeling to resting-state and task-based fMRI data to understand, within the same sample, the neural substrates of CBT effects common across diagnostic categories (MDD and PTSD combined) and specific to each category (MDD or PTSD). Methods: Each patient was scanned twice in a 3T Trio (Siemens) scanner at baseline and after 12-week CBT treatment using the same protocol: a 7-min resting-state scan followed by four runs of emotional conflict task scan. The task was designed to probe the emotional and cognitive control networks. For comparison, 28 age and gender matched healthy controls (HCs) were scanned at baseline. After standard preprocessing, task activation (15 MDD, 16 PTSD) and amygdala intrinsic functional connectivity (17 MDD, 21 PTSD) were computed at baseline and post-treatment. The clinical efficacy of CBT treatment was assessed using Montgomery-Asberg Depression Rating Scale (MADRS), Mood and Anxiety Symptoms Questionnaire (MASQ), and Posttraumatic Stress Diagnostic Scale (PDS). Imaging data were analyzed using voxel-wise LME modeling to examine the common and unique CBT effect on brain indices. Pearson's correlations were conducted to link brain function to symptom improvement. Imaging results were corrected for multiple comparisons using Gaussian Random Field theory ($Z>2.33$, $p<0.005$). Results: Across patients, we found that task activations of the left DLPFC, posterior insula, and doro-medial striatum, and the bilateral IFG and dorsal ACC were normalized after treatment to a magnitude comparable to HCs. In addition, baseline DLPFC activation was correlated with CBT-related changes in MADRS ($r=-0.39$, $p=0.03$, $n=31$): greater baseline DLPFC activity associated with greater improvement in MADRS. The resting-state results

indicated that amygdala functional connectivity with bilateral dorsal ACC and left anterior insula was increased with treatment to a level comparable to HCs. The distinctive neural mechanisms of CBT effects in MDD and PTSD were captured by the group×time interaction. We found that CBT increased in MDD but decreased in PTSD the right DLPFC and IFG activation. In contrast, an opposite pattern was observed for left putamen (increase in PTSD and decrease in MDD). Furthermore, baseline DLPFC activation was correlated with ΔMADRS in MDD ($r=-0.53$, $p=0.04$, $n=15$) but not PTSD. No significant brain-symptom relationships were observed for resting-state data. Conclusions: Our results suggest that normalizing dysfunctions of cognitive control and emotional regulation networks may be a common biological mechanism of CBT efficacy in MDD and PTSD. The dimensional brain-symptom associations observed suggested that one of the brain mechanisms by which CBT improves depressive symptoms is via normalizing the functional deficits in DLPFC. The dimensional brain-behavioral associations observed could potentially serve as an imaging marker to index inter-individual differences in treatment responses. The categorical results suggest that CBT increased activity in cognitive control areas (DLPFC, IFG) in MDD but not PTSD and increased activity in areas related to motivation (putamen) in PTSD but not MDD. Thus, our results provided empirical evidence to support the notion that both dimensional and categorical approaches are valuable and complementary to understanding psychopathology.

RECIPROCAL REGULATION OF MITOCHONDRIAL DYNAMICS AND CALCIUM SIGNALING IN ASTROCYTE PROCESSES

Joshua Jackson

The recruitment and positioning of mitochondria to sites of elevated activity allows neurons, and other cells, to match local energy supply and Ca2+ buffering capacity with demand. Previously, we demonstrated that mitochondria are immobilized in astrocytes in response to neuronal activity, glutamate uptake, and reversed Na+/ Ca2+ exchange. We also demonstrated that neuronal activity increases the probability that mitochondria appose GLT-1 puncta within astrocyte processes. In the present study, we examined the interrelationships between mitochondrial mobility and Ca2+ signaling in astrocyte processes in organotypic cultures of rat hippocampus. We used membrane-tethered genetic Ca2+ indicators (GCaMPs) to monitor spontaneous Ca2+ signals in astrocyte processes. As has been previously described, we observed spontaneous Ca2+ spikes with half-lives of ~1 s that spread ~6 μm and are almost abolished by a TRPA1 channel antagonist. Nearly all of these Ca2+ spikes overlap mitochondria (98%), and 62% of mitochondria are overlapped by these spikes. We investigated whether and how Ca2+ signaling contributes to mitochondrial immobilization. Inhibition of neuronal activity, glutamate uptake, or reversed Na+/Ca2+-exchange all decreased basal [Ca2+] and altered Ca2+ spikes. We show that the Ca2+-adaptor proteins Miro1 and Miro2 are both expressed in astrocytes and that exogenous expression of Ca2+-insensitive Miro mutants nearly doubles the percentage of mobile mitochondria. Expression of Ca2+-insensitive Miro1 had a modest effect on the frequency of these Ca2+ spikes but nearly doubled the decay half-life. The mitochondrial proton ionophore, FCCP, caused a large, prolonged increase in cytosolic Ca2+ followed by an increase in the decay time and the spread of the spontaneous Ca2+ spikes. Photo-ablation of individual mitochondria in astrocyte processes has similar effects on Ca2+. Together, these studies demonstrate a mechanism by which mitochondria are immobilized in astrocytes subsequent to increases in intracellular [Ca2+] where they contribute to the compartmentalization of Ca2+ signals in astrocyte processes. This may also provide a mechanism to coordinate increases in neuronal activity with increases in metabolism in astrocytes.

ACETYL-COA METABOLISM BY ACSS2 REGULATES NEURONAL HISTONE ACETYLATION AND LONG-TERM MEMORY

Philipp Mews

Metabolic production of acetyl-CoA has been linked to histone acetylation and gene regulation, however the mechanisms are largely unknown. We show that the metabolic enzyme acetyl-CoA synthetase 2 (ACSS2) is a critical and direct regulator of histone acetylation in neurons and of long-term mammalian memory. We observe increased nuclear ACSS2 in differentiating neurons in vitro. Genome-wide, ACSS2 binding corresponds with increased histone acetylation and gene expression of key neuronal genes. These data indicate that ACSS2 functions as a chromatin-bound co-activator to increase local concentrations of acetyl-CoA and to locally promote histone acetylation for transcription of neuron-specific genes. Remarkably, in vivo attenuation of hippocampal ACSS2 expression in adult mice impairs long-term spatial memory, a cognitive process reliant on histone acetylation. ACSS2 reduction in hippocampus also leads to a defect in upregulation of key neuronal genes involved in memory. These results reveal a unique connection between cellular metabolism and neural plasticity, and establish a link between generation of acetyl-CoA and neuronal chromatin regulation.

PHOSPHORYLATION OF THE TRANSLATION REPRESSOR, ELF4EBP2, IS THE CRITICAL MEDIATOR OF MEMORY IMPAIRMENTS INDUCED BY SLEEP DEPRIVATION

Jennifer Tudor

Sleep loss produces deficits in hippocampal synaptic plasticity and hippocampus-dependent memory storage. However, the molecular and cellular mechanisms that underlie these effects of sleep deprivation remain unclear. Previous work from our laboratory demonstrated that a prominent effect of even brief periods of sleep deprivation is attenuation of mammalian target of rapamycin (mTOR) signaling in the hippocampus. Specifically, five hours of total sleep deprivation reduces phosphorylated eukaryotic translation initiation factor 4E binding protein 2 (4EBP2) that subsequently leads to impaired protein synthesis. However it is yet to be determined whether restoring downstream mTOR signaling in the hippocampus is sufficient to prevent the cognitive deficits associated with sleep deprivation. To address this important question, we developed an adeno-associated virus (AAV) with a CaMKII alpha promoter fragment to induce expression of mutant phosphomimetic 4EBP2 selectively in excitatory neurons of the hippocampus. Mice were bilaterally injected with phosphomimetic 4EBP2 AAV and mice injected with enhanced green fluorescent protein (eGFP) AAV served as controls. Three weeks after hippocampal AAV infection, mice were trained in the hippocampus-dependent object place recognition task. Afterwards, mice were sleep deprived for five hours or left undisturbed in their home cage. We found that hippocampal expression of phosphomimetic 4EBP2 prevented the memory deficits associated with sleep deprivation in the object place recognition task. We also expressed a mutant phosphodefective 4EBP2 in the hippocampus via AAV infection and these mice had memory impairments, both with and without sleep deprivation. These findings indicate that phosphorylation of 4EBP2 in the hippocampus is the critical component underlying the memory deficits associated with sleep deprivation in hippocampus-dependent learning tasks. Furthermore, this study defines the molecular mechanism by which loss of sleep impairs cognitive processes and highlights a vital role for translation and mTOR activation on long-term memory formation.

"TOO MUCH OF A GOOD THING?" A HEIGHTENED BRAIN RESPONSE TO 6 SECOND COCAINE VIDEO CUES PREDICTS POOR DRUG USE OUTCOMES IN TREATMENT-SEEKING COCAINE PATIENTS

Anna Rose Childress

Background: From an evolutionary perspective, individuals with a greater sensitivity to signals for reward (food, sexual opportunity, attachment) had a survival advantage – turning both animals and humans into exquisite "reward detectors". Ironically, our highly-conserved, survival-driven sensitivity to reward signals may have a potential dark side. For addicted individuals, a heightened sensitivity to drug reward cues could be "too much of a good thing", putting them at greater risk for relapse. We have hypothesized that individual differences in relapse vulnerability may be traced to two, interactive, brain systems: the brain's incentive motivational ("GO!") circuitry, triggered by rewards and their signals, and the brain's modulatory ("STOP!") circuitry, responsible for inhibiting and managing the pull of incentive stimuli. When encountering cues signaling a drug reward, either an over-responsive "GO!" circuit, or an under-responsive "STOP" circuit, or both, could increase the likelihood of relapse. We directly tested this hypothesis in a new cohort of cocaine-addicted individuals, using a task that probed brain responses both while cocaine patients simply watched drug reward cues, and while they actively attempted to inhibit their responses to the cues. We predicted that individuals with poor (future) drug use outcomes would have a stronger brain response (especially in motivational circuitry) to the cocaine cues, and that they would not be able to inhibit this response. Methods: Our participants were selected from a new cohort (n=39) of cocaine-dependent patients in large study focused on brain predictors of relapse. Each individual received inpatient stabilization followed by a functional magnetic resonance imaging (fMRI) session with several probes. The inpatients were then discharged into 12 weeks of outpatient treatment, with twice weekly urine samples. For the current outcome-based comparisons, we selected two phenotypic extremes, "POOR" outcome individuals (more than 90% urines cocaine positive/missing; n=12) and "GOOD" outcome individuals (30% or fewer urines cocaine postiive/missing, n=9). Each patient underwent BOLD (Blood Oxygen-Level-Dependent) fMRI imaging to examine the brain response to a pseudo-random series of 6-second video clips. The videos represented three conditions (6 clips per condition): WATCH ("Just watch" the cocaine video), DOWN (Please try to reduce your feelings to the cocaine video), or NEUTRAL ("Just watch" the Neutral video). Data were smoothed, normalized, realigned and batch-analyzed within SPM 8, using canonical HRF as the basis function. Pre-planned contrasts compared the brain response to the WATCH vs. the NEUTRAL condition, and

to the DOWN vs. the NEUTRAL condition, for the whole task, and for the first and second halves of the task (to allow us to examine for change in response as the task progressed). Statistical parametric (t) maps for the second-level (group) analyses (whole group, and for the two phenotypic extremes “GOOD” and “POOR”) were thresholded at 2<t<5 for display. Results: For the WATCH (cocaine video) vs. NEUTRAL comparison, cocaine-addicted individuals who would proceed to a POOR outcome showed a dramatic, widespread brain activation that included not only the classical nodes for motivational processing (e.g., ventral tegmental area, amygdala, ventral striatum/pallidum, medial orbitofrontal cortex, mOFC), but also strong activation in the dorsal cortex, the posterior cingulate cortex and visual cortices. In stark contrast, cocaine patients who would later go onto GOOD outcomes had remarkably “quiet” brains during the cocaine video. Results from the “DOWN” (cocaine video) vs. Neutral condition generally echoed those in the “WATCH” analyses: the POOR outcome group showed massive activation in the mesolimbic circuitry and the visual cortices during “DOWN” (and this activation pattern was even more striking in the second half of the task), while the GOOD outcome group showed much “quieter” brains with only minor activations (in ventral striatum, and in dorsolateral prefrontal cortex, DLPFC, a modulatory region) by the final half of the DOWN task. Discussion: These findings offer a clear demonstration that drug cue-provoked brain responses may be able to predict future relapse in addicted individuals. The difference between the two phenotypic extremes was dramatic, with the POOR outcome group showing an impressive, widespread brain response to the drug cues...even when attempting to inhibit. The results have several implications. From an evolutionary perspective, individuals with heightened reward sensitivity may be very "fit" -- but they could be at a disadvantage in contemporary environments offering easy access to energy-dense foods, to sexual partners with transmissible infections, and to compelling drugs of abuse. From a mechanistic perspective, these data suggest that the brain response to drug cues in incentive motivational (GO!) circuits is likely to be an important relapse substrate. From a practical perspective, the brain response to drug cues may be a very useful research tool, allowing us to screen candidate medications for their ability to engage relapse-relevant brain targets -- offering new hope for individuals with "too much of a good thing". Supported by: Commonwealth of Pennsylvania CURE Addiction Center of Excellence: Brain Mechanisms of Relapse and Recovery, and NIH/NIDA U54DA039002.

STRUCTURAL CORRELATES OF INDIVIDUAL DIFFERENCES IN MOTOR SEQUENCE LEARNING

Ari Kahn

Human skill learning is a complex phenomenon that involves the fine-scale coordination of disparate cortical and sub-cortical regions. This coordination critically depends on the effective transmission of information across white matter tracts, which link distant brain regions in cortico-cortical networks and cortico-subcortical loops. We assessed the influence of differences in white matter connectivity on learning rates. We presented subjects with a motor learning tasks reliant on visual cues, and collected four diffusion scans on subjects over a 6-week period while they practiced the task. We estimated learning rate by fitting a double exponential curve to each subject. We then analyzed tractography-based connectivity, and found that increased visual white matter connectivity predicts subject learning rate. Moreover, the influence of connectivity between visual and motor regions, which have minimal direct white matter connectivity, could be observed when analyzed with network-based tools. Our results demonstrate that the combination of diffusion imaging and tractography-based connectivity in subjects can provide predictive information about task-specific learning.

DOUBLE DISSOCIATION OF VALENCE AND VIVIDNESS EFFECTS ON THE DORSAL AND VENTRAL DEFAULT MODE NETWORK

Trishala Parthasarathi

Recent work has shown that the default mode network (DMN) is activated when imagining the future. However, it is unknown whether different aspects of imagination engage different nodes of the DMN. Here, we test how the vividness, valence, and temporal distance of an imagined event differentially modulates BOLD activity. Twenty-four people were scanned using fMRI while imagining scenarios manipulated for vividness, valence, and temporal distance. Subjects rated each scenario for vividness and valence. We analyzed our neuroimaging data using the general linear model; during the imagination period, we included separate regressors for comparing scenarios that were high versus low in vividness, high versus low in valence, and high versus low in temporal distance. A region-of-interest analysis was also conducted using dorsal and ventral DMN masks obtained from a previous study. At the whole brain level, we found increased BOLD activity in the vmPFC, VS, and medial temporal regions when participants were imagining. Precuneus and hippocampus had increased activity for more vivid scenarios compared to less vivid scenarios, and greater activity was seen in the vmPFC and VS for positive scenarios compared to negative scenarios. ROI results confirmed that valence modulates activity in the dorsal, but not ventral DMN, while vividness modulates activity in the ventral, but not dorsal DMN. These results show that different aspects of imagination differentially modulate separate nodes of the default mode network.

NEURAL CODING OF OBJECT KNOWLEDGE IN PERIRHINAL CORTEX

Amy Price

Many studies have examined object knowledge by studying the neural representation of object categories (e.g., tools versus animals), which often broadly differ on coarse property features, such as shape, size, and texture. However, little is known about the neural mechanisms for encoding the fine-grained semantic attributes of specific objects within a semantic category. For example, how do we know that a red apple is conceptually more similar to a green apple than to a blue apple? Here, we address this question by using a novel stimulus set that allowed us to leverage the natural statistics of object color information to investigate a neural code for object meaning. In an fMRI experiment, 16 subjects viewed images of objects that were systematically manipulated in color while performing an unrelated object detection task. The stimuli included three categories of specific objects (apples, leaves, and roses). The objects were each presented in five different colors (red, pink, blue, green, and yellow). For each object set, we created a semantic-similarity model based on the co-occurrence frequencies of color-object combinations (e.g., "yellow apple" from a large lexical corpus). This model predicts that "red apple" and "green apple" would have more similar neural representations than "red apple" and "pink apple" in brain regions that code high-level combinatorial information about object concepts. The semantic-similarity models were unique for each object category used in the experiment, and were orthogonal to perceptual models for shape or color similarity alone. Using representational similarity analysis of the multi-voxel patterns, we found that perirhinal cortex was the only region that significantly correlated with the semantic-similarity model ($p<0.01$, corrected), while earlier ventral visual regions correlated with the color similarity model and shape similarity model (V4 and LOC, respectively, $p<0.01$). Next, we proposed that a key function of these semantic codes is to provide a common understanding of object meaning across individuals. For example, my stored knowledge of the familiar object "red apple" should be similar to yours if we are to communicate and coordinate our behaviors. This predicts a specific functional architecture: neural codes in this high-level semantic region should be structured to provide a common ground between observers of the visual world. To test for this proposed organization, we hyper-aligned each subject's data to a common, high-dimensional space (Haxby et al., 2011). We hypothesized that within perirhinal cortex, inter-subject similarity would track the semantic typicality of the objects. Indeed, we found that perirhinal cortex was unique in containing population codes for which inter-subject similarity correlated with object typicality ($p<0.01$, corrected). For example, a typical object like "red apple" has a more similar neural instantiation across individuals than a less typical object like "blue apple." Our results suggest that perirhinal cortex encodes combinatorial information that underlies real-world knowledge of objects and may instantiate a neural "common ground" for object meaning across individuals.

A ROLE FOR NEUROPEPTIDE SIGNALING IN REGULATING C. ELEGANS RESPONSE TO ANOXIA

Shachee Doshi

The nematode C. elegans generally prefers 8-12% environmental oxygen, but frequently encounters much lower oxygen microenvironments as it dwells in soil and rotting fruit. It responds to hypoxia and anoxia using distinct molecular pathways, and while the genetic regulation of the hypoxic response has been well characterized in the worm, less is known about the response to anoxic environments. Here, we find a novel role for neuropeptide signaling in regulating a specific response to anoxia using genetic manipulations afforded by this model organism. Loss of function mutants in neuropeptide maturation (egl-3) as well as neuropeptide release (unc-31) have a significant survival advantage after 48hr anoxia compared to wild-type N2 animals. Tissue-specific loss of egl-3 demonstrates that the nervous system is necessary for this effect, confirming that neuropeptides, and not other peptidergic hormones, mediate this regulatory response. This response is independent of the canonical insulin-signaling pathway that is known to protect the worm from a variety of stresses. It is also independent of the hypoxia-inducible factor 1 (hif-1), a transcription factor known to mediate a protective response to hypoxia, thus confirming that distinct pathways regulate responses to hypoxia and anoxia. The role of neuropeptide signaling is also specific to anoxic stress as it does not protect against other stressors such as heat or ER stress. Others have reported that the homolog of yeast longevity assurance gene 2 (hyl-2), a ceramide synthase, protects C. elegans from anoxia as hyl-2 mutants are very poor survivors of anoxic stress. We made double mutants to test whether loss of neuropeptide secretion is dependent on hyl-2 for its protective effect. Our results indicate that hyl-2 is indeed necessary for the protective effect conferred by loss of neuropeptide signaling. Ongoing work aims to describe the mechanistic link between unc-31/egl-3 and hyl-2 in regulating this response. We are also working to define the specific neuropeptide(s) and the cell-types responsible for neuropeptide-mediated death under anoxia. This work sheds light on a novel and specific pathway regulating the response to severe oxygen deprivation, and could have implications for a variety of human conditions including, among others, hypoxic ischemic encephalopathy and stroke.

AGE OF ONSET OF MARIJUANA USE AND NEURAL RESPONSES TO BACKWARD-MASKED MARIJUANA CUES: SHIFTS IN STRIATAL ACTIVITY

Nathan Hager

Age of onset of marijuana use and neural responses to backward-masked marijuana cues: shifts in striatal activity Authors: Nathan Hager, Reagan R. Wetherill, Yasmin Mashhoon, Kanchana Jagannathan, Teresa R. Franklin Marijuana (MJ) is one of the most commonly used illicit drugs, and as the perceived risk of using MJ continues to decline, initiation of MJ use is occurring at even younger ages. Early age of onset of MJ use is concerning because adolescence is a critical period of neuromaturation and research suggests that MJ use is associated with cognitive deficits and changes in brain structure and function. Thus, we hypothesize that individuals with an early onset of MJ use (regular use prior to age 16) will exhibit a different pattern of reward-related neural responses to MJ cues compared to individuals with a later age of onset (age 16 or later). Using functional MRI and an event-related blood oxygen level-dependent (BOLD) backward-masking task, we compared neural responses to backward-masked MJ cues to neutral cues in treatment-seeking, cannabis-dependent adults (N = 44; 27 males). Individuals were grouped as early onset (EOs, < 16; n = 16) or late onset (LOs, ≥16; n = 27). EOs and LOs were demographically similar and did not differ in age of onset for other substances (e.g., alcohol, nicotine). EOs and LOs differed in lifetime MJ consumption (EOs=16.6 ± 4.8 eighth yrs; LOs=7.9 ±1.8 eighth yrs; $p < .05$)*. Unique differential patterns of striatal activation, ventral in LOs and dorsal in EOs, were elicited by backward-masked MJ cues compared to neutral cues. In drug cue- and reward-related circuitry, ventral striatal activity is critical for incentive-based learning and mediating goal-directed behaviors whereas dorsal striatal activity is related to processing action-reward consequences (Tricomi et al. 2004), anticipating reward (O'Doherty et al., 2002) and regulating habitual drug-seeking behavior (Canales, 2005). Current BOLD findings, likely influenced by years of MJ exposure in each group, indicating ventral striatal incentive goal-directed activation in LOs and dorsal striatal habit-based MJ-seeking behavior in EOs are among the first to parallel previous preclinical studies in exhibiting a shift between goal-directed and habitual drug-related processing (Everitt and Robbins, 2005). Future study is needed to determine whether the choice to use drug at an earlier age is associated with a faulty reward system or if MJ use at an early age affected normal development. Further, the effects of sex on reward system function deserve further exploration. *eighth years calculation: grams per day/3.5 X years of use. 3.5 is # of grams in an eighth of an ounce of MJ– a typical amount of MJ consumed per day. This measure provides a measure of lifetime consumption that is equivalent to that of pack years in cigarette smokers.

ASTROCYTIC MITOCHONDRIA UNDERGO DELAYED FRAGMENTATION AND DEGRADATION IN RESPONSE TO AN IN VITRO MODEL OF ISCHEMIA/REPERFUSION INJURY

John O'Donnell

Recent studies have demonstrated that mitochondria are found throughout astrocytic processes and shape Ca2+ signaling. Mitochondria and Ca2+ signaling in astrocytic processes have not been examined in models of ischemia/reperfusion injury. In the present study, we biolistically transfected astrocytes in rat hippocampal slice cultures to facilitate fluorescent confocal microscopy, and subjected these slices to transient oxygen/glucose deprivation (OGD) that causes delayed excitotoxic death of CA1 pyramidal neurons. This insult caused a transient increase in the number of mitochondria in astrocytic processes with an ~50% decrease in the average size of mitochondria and the fraction of process occupied by mitochondria. These effects were blocked by iGluR antagonists and mimicked by N-methyl-D-aspartate. Mitochondrial loss was associated with increased co-localization of mitochondria and the autophagosome marker LC3B. Blocking glutamate uptake with (3S)-3-[[3-[[4-(trifluoromethyl)benzoyl]amino]phenyl]methoxy]-L-aspartate (TFB-TBOA) increased neuronal loss after OGD or NMDA, but surprisingly blocked the loss of astrocytic mitochondria completely. Two calcineurin inhibitors, cyclosporin-A or FK506, also attenuated the loss of mitochondria. Using the high-affinity genetic Ca2+ indicator Lck-GCaMP-6S, we observed two types of Ca2+ signals; the first occupied the cytoplasm surrounding mitochondria ('mitochondrially-centered') and the second traversed the space between mitochondria ('extra-mitochondrial'). The spatial spread, kinetics, and frequency of these events were different. The amplitude of both types was doubled and the spread of both types changed by ~2-fold 24 hrs after OGD. These effects were blocked by cyclosporin-A. In summary, transient OGD caused a delayed loss of mitochondria via mitophagy and dramatically increased Ca2+ signaling in astrocytic processes.

COMMON AND UNIQUE NEURAL CIRCUIT DISRUPTION IN COGNITIVE AND EMOTIONAL FUNCTIONING ACROSS PSYCHIATRIC DISORDERS

Benjamin Rosenberg

Background: In a recent meta-analysis of gray matter volume in Axis I disorders (Goodkind et al., 2015), we identified a transdiagnostic pattern of tissue loss specific to and prominent in the dorsal anterior cingulate (dACC) and bilateral anterior insula, key nodes in the salience network. The current meta-analyses examined whether these nodes are additionally linked to transdiagnostic deficits in brain activation during cognitive as well as emotional functioning. Methods: Studies on cognitive (n=283) and emotional (n=252) functioning including patients across a range of Axis I diagnoses (bipolar, depression, anxiety, substance abuse/dependence, schizophrenia, psychosis) and matched control participants were submitted to meta-analysis. Cognitive/executive function or emotion provocation tasks during functional brain imaging that reported significant whole-brain, voxel-wise group differences in stereotaxic space were included. Meta-analysis utilized peak voxel coordinates for activation likelihood estimation of patterns reflecting patient hyper- or hypo-reactivity in relation to control participants. Results: During cognitive processing tasks, transdiagnostic patient hypoactivation was evident in anterior insula and dorsal anterior cingulate as well as left dorsolateral prefrontal cortex (dlPFC) and bilateral intraparietal sulci. The opposite pattern of patient hyperactivation emerged transdiagnostically in the mid-cingulate cortex/pre- supplementary motor area. Diagnostic-specific effects were observed unique to the left dlPFC due to pronounced hypoactivation in schizophrenia. During emotion processing, transdiagnostic effects emerged for patient hyperactivation in bilateral insula, left amygdala, and ventromedial prefrontal cortex, whereas dACC hyperactivation was specific to non-psychotic disorders. Conclusions: Consistent with prior evidence of structural integrity disruptions, key nodes in the salience network showed impaired activation during a range of executive and emotional functioning tasks—across Axis I disorders. The salience network is implicated as a common pathway to cognitive and emotional dyscontrol in psychopathology and potentially as a powerful common target for therapeutic intervention.

INDIVIDUAL DIFFERENCES IN THE PROCESSING OF ANTI-SMOKING PUBLIC SERVICE ANNOUNCEMENTS: AN IMAGING GENETICS STUDY

Zhenhao Shi

Background: Televised public service announcements (PSAs) play an important role in anti-smoking campaigns. However, their effectiveness is moderate at best. Understanding individual differences in the processing of these PSAs is critical for increasing their effectiveness. The 10-repeat allele of the variable number tandem repeat (VNTR) polymorphism of the dopamine transporter gene (DAT1) has been associated with greater risk of addiction and information processing deficits. We hypothesized that smokers with the 10-repeat allele of the DAT1 polymorphism may respond better to PSAs with high message sensation value (MSV), which is an aggregate measure of the sensory intensity of audio and visual features of PSAs. Methods: Using functional magnetic resonance imaging (fMRI), we examined the neural response to High- and Low-MSV PSAs in 53 smokers (23 female, age=18–49) who were either homozygous for the DAT1 10-repeat allele (High-Risk, N=26) or with nine- or fewer-repeat alleles (Low-Risk, N=27). Urine cotinine levels were tested before and 4 weeks after the fMRI session. Results: Compared to the Low-Risk group, the High-Risk group showed reduced visual and auditory cortical response to PSAs in general (x/y/z=0/-82/-2 & -63/-25/10, corrected $p<0.01$), and reduced right temporoparietal junction (rTPJ) response to Low-MSV PSAs in particular (x/y/z=48/-43/34, corrected $p<0.01$). These neural activity mediated the effect of DAT1 genotype on longer-term smoking behavioral indexed by urine cotinine levels ($p<0.05$). Conclusions: Our findings suggest that DAT1 genotype contributes to the individual variability in the brain and behavioral processing of anti-smoking PSAs.

Sheng Tang

DISTINCT MORPHOLOGIC AND FUNCTIONAL CHANGES UNDERLIE DIVERGENT PHENOTYPES IN CDKL5 CONDITIONAL KNOCKOUT MICE

Sheng Tang, Barbara Terzic

CDKL5 is a devastating neurodevelopmental disorder characterized by early-onset epilepsy, severe intellectual disability, and autistic features. Constitutive mice bearing a loss-of-function mutation in the X-linked gene cyclin-dependent kinase-like 5 (*Cdkl5*) recapitulate aspects of human symptomatology. To probe the cellular and developmental origins of CDKL5 disorder-related phenotypes, we have constructed a *Cdkl5* conditional allele and used it to generate cell-type specific knockout mice lacking CDKL5 in either excitatory or inhibitory forebrain neurons. Behavioral characterization suggests an apparent segregation of autistic and epileptic phenotypes, while cell morphologic and synaptic studies indicate distinct patterns of neuronal dysfunction. In summaries, our studies have begun to delineate cell-type specific contributions to CDKL5 disorder-related phenotypes.

ROUNDABOUT2 AND EXOTOSIN-LIKE 3 PROMOTE TARGET SPECIFIC PERIPHERAL NERVE REGENERATION IN VIVO

Patti Murphy

Following injury, axons of the peripheral nervous system regenerate, but only a small fraction reinnervate their original targets. Instead, regenerating axons grow too slowly to reach their original targets before supporting Schwann cells degenerate, and/or axons grow in the wrong direction and innervate inappropriate targets. To decipher the cellular and molecular mechanisms that guide regenerating axons toward their original targets, we study target selective regeneration of spinal motor nerves in larval zebrafish. Each nerve is composed of a ventral and a dorsal branch that diverge at a stereotyped choice-point. We have recently shown that following laser mediated nerve transection, axons of the dorsal branch select their original trajectory with high fidelity (>70%; Issacman-Beck et al, Neuron, 2015). Moreover, we find that slit1a mRNA is upregulated in denervated Schwann cells distal to the lesion site, suggesting a role for slit/robo signaling in directing regenerating dorsal axons. To directly probe the in vivo role of slit/robo signaling, we examined mutants for the slit receptor robo2. While robo2 appears dispensable for motor nerve development, we find that regenerating dorsal axons in robo2 mutants frequently (45%) fail to regenerate towards their original targets, and instead extend along ectopic trajectories. Interestingly, we find that exotosin-like 3 (extl3) mutants display a similar phenotype with higher frequency (61%). Extl3 modifies heparan sulfate proteoglycans, which are known to stabilize slit/robo binding. Finally, we find that robo2 and extl3 function specifically in directing axons of the dorsal nerve branch – regenerating axons of the ventral nerve branch select their original targets with high fidelity in robo2 and extl3 mutants. Thus, extl3 and robo2 mediate pathway specific axon guidance during peripheral nerve regeneration. We will present ongoing work on how robo2 and extl3 promote target specific regeneration.

ALL-OPTICAL PHYSIOLOGY VIA SIMULTANEOUS TWO-PHOTON CALCIUM IMAGING OPTOGENETIC STIMULATION OF A RED-SHIFTED CHANNELRHODOPSIN

Ethan Goldberg

Achieving a greater understanding of the logic of neural circuit function and the basis of circuit dysfunction in disease will require the continued improvement of experimental tools capable of monitoring and manipulating the nervous system. Two-photon microscopy of calcium-sensitive probes allows imaging of the activity of many neurons at high speed and with cellular level resolution including relatively deep in the light-scattering environment of brain tissue. Optogenetics utilizes light-sensitive molecules to manipulate neuronal activity remotely, also with cellular-level resolution, and millisecond temporal precision. Combining these techniques allows for an unprecedented ability to investigate mechanisms of neural circuit function, and there has been recent and rapid progress in this arena. Here, we demonstrate the simultaneous combination of two-photon calcium imaging of the genetically-encoded indicator GCaMP6f with one-photon photostimulation of the red-shifted channelrhodopsin variant ChrimsonR in cultured neurons and in acute brain slices in vitro based on simple modifications to a standard two-photon imaging system. This technique will facilitate the ability to test the role of defined subclasses of neurons in circuit-level phenomena, or probe the connectivity between cell types, in an “all-optical” fashion.

PROBING THE OLFACTORY CODE USING ANTAGONISTS

Marissa Kamarck

The olfactory system recognizes each odor using a unique subset of odorant receptors. Although the specific pattern of odorant receptors activated by an odorant code for the odorant's identity, there are few, if any, explicit predictions relating odorant receptor activity patterns to olfactory perception. Given that the receptor activation patterns encode the odorant identity and intensity, antagonizing these receptors should alter perception of the odorant. Here we targeted a trace-amine associated receptor (TAAR) due to previous work indicating that genetic knockout of a single TAAR eliminated innate aversion to a predator odor (Dewan et al., 2013). Human TAAR5, activated by trimethylamine (TMA), is the only human TAAR with a published ligand (Wallrabenstein et al., 2013). Using a cell-based luciferase assay, we first identified an in vitro antagonist for hTAAR5 ($p<0.0001$), and then tested the antagonist in a psychophysical paradigm. Using an olfactometer, we presented subjects with one of the following odors: TMA alone, the antagonist alone, or TMA with the antagonist. Subjects were asked to rate the intensities of the odor qualities in each mixture. TMA-alone and antagonist-alone were not different in intensity ($p = 0.217$). The antagonist decreased the perceived intensity of TMA relative to both TMA-alone ($p<0.01$) and TMA mixed with an intensity-matched control odor ($p<0.05$). Antagonist odors promise to be a powerful tool for examining the contribution of individual receptors to odor perception.

HUMAN PATHWAY TO AMYGDALA INHIBITION AND DISRUPTION IN PTSD

Desmond Oathes

Combining non-invasive transcranial magnetic stimulation (TMS) with functional MRI allows an unprecedented degree of control in probing neural circuits in awake live humans. By inducing activation at a key node in a neural network, the downstream causally related communication partners of that brain area can be functionally elucidated. This strategy has implications across cognitive neuroscience but also specifically in defining novel neurotherapeutics. Based on neural tracing studies in non-human primates and human functional imaging studies, we hypothesized the existence of a pathway from the ventrolateral prefrontal cortex to the amygdala in humans. By interleaving fMRI recordings with single TMS pulses, we discovered for the first time evidence of a causal pathway from the vlPFC to the amygdala in humans. Using a large high quality diffusion imaging dataset from the Human Connectome Project, we found evidence for a direct structural pathway between our vlPFC stimulation site and the amygdala. Stimulating this prefrontal site led to a decrease in amygdala response among healthy individuals but a lack of response in patients diagnosed with posttraumatic stress disorder (PTSD). Our resting fMRI data in the same participants also suggested intact communication in this circuit among healthy participants that was not present in PTSD patients. The relative disruption in the circuit for patients was related to their reported PTSD symptom levels as well as predicted their clinical outcomes in response to psychotherapy (prolonged exposure therapy). In summary, we here establish a causal link between the vlPFC and amygdala established in vivo for the first time in humans. Our data indicate that the vlPFC can effectively down regulate the amygdala in healthy individuals using non-invasive surface TMS. TMS to the vlPFC also revealed a PTSD disruption in down regulating the amygdala that was an abnormality linked to symptomatology and predictive of treatment outcome. Our findings underscore the vlPFC-amygdala circuit in humans as a focus for cross-sectional studies as well as novel brain based neuropsychiatric interventions.

STRESS INCREASES ALCOHOL SELF-ADMINISTRATION VIA EXCITATORY GABA NEUROTRANSMISSION IN THE VTA

Alexey Ostroumov

Although stress has been shown to promote alcohol intake in humans, the synaptic and circuit-level mechanisms contributing to this interaction remain largely unknown. We examined the effect of a single restraint stress on alcohol self-administration and on alcohol-induced dopamine (DA) signaling via in vivo microdialysis and in vitro electrophysiology. Application of restraint stress 15 hours prior to alcohol exposure significantly increased alcohol self-administration over many days and decreased alcohol-induced DA signaling in the nucleus accumbens. In vitro patch electrode recordings showed that this decreased DA signaling was mediated by increased GABAergic inhibition onto DA neurons in the ventral tegmental area (VTA). Following stress, local VTA GABA neurons showed a higher firing rate in response to alcohol compared to non-stressed controls. The increase in alcohol-induced GABA neuron firing after stress was blocked by bath application of picrotoxin, suggesting GABAA-mediated excitation. In support, we found that stress caused a positive shift in the reversal potential of GABAA receptors located on VTA GABA neurons, an effect that could contribute to cell excitability in the presence of alcohol. To test this hypothesis, we blocked GABAA receptor-mediated excitation with acetazolamide. Acetazolamide prevented the effect of stress on alcohol-induced neurotransmission in vitro and in vivo. These findings provide evidence that acute stress switches GABAA receptor function from inhibitory to excitatory in the presence of alcohol, which significantly alters DA neuron signaling and may contribute to increased alcohol self-administration.

WHITE MATTER CONNECTIVITY SUPPORTS INCREASING DIVERSITY OF NEURAL DYNAMICS ACROSS NORMATIVE NEURODEVELOPMENT

Evelyn Tang

The human brain is able to support a wide range of cognitive functions via the coordinated activity of multiple regions. Such coordination can come in many flavors, including the control of large-scale processes, the synchronization of two or more brain regions, and the tuning of activity patterns across length scales. The role of white matter connectivity in supporting these different coordination types is not well understood, nor is it easy to quantify how such structure emerges across development. Here we use a network representation of diffusion imaging data to show that the pattern of white matter connectivity changes appreciably with age, displaying a global increase in controllability and associated decrease in global synchronizability. These trends emerged from an ensemble of networks obtained from diffusion tensor imaging of 880 healthy individuals from ages 8 to 22. In addition to these global trends, we also observed pronounced regional differentiation in which regions of high control become super-controllers whose influence only extends locally to short length-scale synchronization. Conversely, regions of low control in executive areas become even less influential, but whose influence extends broadly to long length-scale synchronization. Our results quantify the range of supported dynamics and regional specialization in the brain, which significantly increase with age. These analytical methods allow the relating of disparate cognitive systems to different types of controllability and oscillatory patterns – indicating how structure can facilitate rhythms and more general dynamics in the human brain.

TRANSLATING RNAI THERAPY FOR SPINOCEREBELLAR ATAXIA TYPE 1

Megan Keiser

Spinocerebellar ataxia 1 (SCA1) is among a group polyglutamine expansion diseases and is characterized by cerebellar ataxia and neuronal degeneration in the cerebellum and brainstem. Currently, there are no effective treatment strategies for this disease. RNA interference (RNAi) is a naturally occurring process that mediates gene silencing and is currently being investigated as a therapy for dominant diseases such as SCA1. Previously, we used AAV vectors to deliver RNAi triggers to transgenic and knock-in mouse models of SCA1 and noted improved neuropathological, motor phenotypes and transcriptional changes. We have also completed studies in non-human primates (NHPs) evaluating the biodistribution, safety and efficacy of vector delivery to the deep cerebellar nuclei (DCN) in NHPs. We next performed dosing studies in pre- and post-symptomatic mice to identify the lowest efficacious dose, the highest tolerated dose. For this, groups of pre-symptomatic mice were given 1 of 4 doses at 5 weeks of age and motor function assayed after symptom onset for untreated mice (34 weeks of age) and immediately sacrificed for post-necropsy analysis. We identified a ceiling dose that conferred toxicity, a low dose that had no effect, and two doses that prevented phenotypic rotarod deficits relative to control injected SCA1 littermates. Concurrently, post-symptomatic mice were injected at 12 weeks of age at 3 escalating doses. Motor function tests at rotarod at 20 weeks of age identified a dose that not only prevented further deficit but significantly improved performance relative to baseline performance. Thus, our AAV-mediated delivery of RNAi to the SCA1 model can reverse motor impairment in mice, and is scalable to nonhuman primates, two important considerations in advancing this therapy to the clinic.

IDENTIFICATION OF GUIDANCE MOLECULES THAT REGULATE AXON TARGETING OF OLFACTORY SENSORY NEURONS USING RNASEQ

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The axonal projection of olfactory sensory neurons (OSNs) from the nasal epithelium to the olfactory bulb is an attractive system in which to study the development of complex circuitry. The two major subclasses of OSNs express different markers, OMP and TRPC2, and project to distinct, non-overlapping protoglomerular targets in the developing olfactory bulb. The mechanism governing the initial targeting of axons is largely dependent on axon guidance molecules. Only a handful of guidance molecules have so far been identified to play a role in early targeting through studies employing candidate gene approaches. In this study, we have used an unbiased RNASeq approach to identify axon guidance factors that are expressed in the olfactory sensory neurons of 48hpf zebrafish. We have identified receptor-ligand pairs that are differentially expressed between the OMP and TRPC2 subclasses of sensory neurons. We have also identified adhesion molecules and Ig superfamily molecules that are expressed differentially between these subclasses. Together, these molecules could regulate the differential targeting of OMP and TRPC2 neurons to non-overlapping protoglomerular targets in the developing olfactory bulb. Using single-cell RNASeq of OMP sensory neurons, we can further detect two different transcription profiles of guidance factors in neurons that project to the central vs dorsal zones.