2016 MISFN Meeting Schedule:

8:30 - 9:00  Registration and Continental Breakfast (Brody Hall Foyer, 134,136,138)
9:00 - 10:15 Poster Session A (Brody Hall 134,136,138)
10:15 - 10:45 Break – visit our vendors!
10:45 - 12:00 Poster Session B (Brody Hall 134,136,138)
12:00 - 1:00 Lunch (Brody Square)
1:00 – 2:00 Business Meeting (Brody Auditorium)
2:00 – 2:30 **Founder’s Award Speaker: Christopher J. Fitzpatrick** (Brody Auditorium)
   “Thalamic mast cells are associated with sign-tracking behavior in rats”
2:30 – 3:00 Break
3:00 – 4:00 **Keynote Speaker: Dr. Mriganka Sur** (Brody Auditorium)
   “The functional logic of cortical circuits”
4:00 - 4:30 Awards and Adjournment (Brody Auditorium)
Founder’s Award

This award is in honor of Montford F. Piercey and Duncan McCarthy for their contributions to organizing our chapter. This year’s recipient is Christopher J. Fitzpatrick, a graduate student in the laboratory of Dr. Jonathan D. Morrow in the Neuroscience Graduate Program at University of Michigan.

Thalamic mast cells are associated with sign-tracking behavior in rats

Christopher J. Fitzpatrick and Jonathan D. Morrow

1 Neuroscience Graduate Program, University of Michigan, Ann Arbor, MI 48109, USA
2 Department of Psychiatry, University of Michigan, Ann Arbor, MI 48109, USA

Mast cells are resident immune cells in the thalamus that can degranulate and release hundreds of signaling molecules (i.e., monoamines, growth factors, and cytokines) both basally and in response to environmental stimuli. They contribute 90% of thalamic histamine in rodents, and histamine has been implicated in appetitive behaviors and motivational processes. Interestingly, mast cell numbers in the brain also show immense individual variation in both rodents and humans. In the present study, we used a Pavlovian conditioned approach (PCA) procedure to examine whether mast cells are associated with the attribution of incentive-motivational value to reward-related cues. During the PCA procedure, a lever response-independently predicts the presentation of a food pellet into a magazine, and over training three conditioned responses (CRs) develop: sign-tracking (lever-directed CR), goal-tracking (magazine-directed responses), and an intermediate response (both CRs). In Experiment 1, we measured thalamic mast cell number/activation using toluidine blue and discovered that sign-trackers have increased total and degranulated (activated) mast cells. Moreover, degranulated mast cells positively correlated with PCA behavior across phenotypes (i.e., rats with higher sign-tracking had higher numbers of degranulated mast cells). In Experiment 2, we infused a mast-cell inhibitor, cromolyn (200 μg/rat; i.c.v.), immediately before five daily PCA training sessions and found that mast cell inhibition impairs the acquisition of sign-tracking behavior. Taken together, these results demonstrate that thalamic mast cells contribute to the attribution of incentive-motivational value to reward-related cues and suggest that mast cell inhibition may be a novel pharmacotherapy to treat addiction.
The computational power of the human brain derives from its neuronal wiring. The brain has about 100 billion neurons, and each neuron connects with hundreds of other neurons via thousands of synapses. Thus, the brain has over 100 trillion ($10^{14}$) synapses - this staggering number of connections, together with the precision of wiring between neurons, enables the complexity of brain processing. Neuronal circuits and networks are the engine of the brain, for they transform simple inputs into complex outputs. Novel technologies are transforming the analysis of brain circuits. Research in our laboratory demonstrates how specific circuits of the cerebral cortex mediate feature selectivity in vision, temporal coding by neuronal populations, and dynamic routing of information between cortical areas. The logic of these circuits reveals fundamental principles of information processing underlying behavior and cognition, and lays the groundwork for rich computational, theoretical and mechanistic analyses of normal and abnormal brain function.
## MiSFN 2016 Poster Assignments

### Cognition and Behavior

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Epidemiological data suggest that body mass index and obesity are strong risk factors for anxiety and depression in humans. In rodents, diet-induced obesity (DIO) produces depression-like behaviors in the forced swim and sucrose preference tests, but the mechanism underlying this effect is poorly understood, and relatively few studies have examined anxiety-like behaviors in rodent obesity models. Here we used a selectively bred rat model to examine basal and obesity-induced differences in anxiety-like behaviors in obesity-prone (OP) versus obesity-resistant rats (OR). Anxiety-like behavior was measured in the elevated plus maze (EPM) and in the open field (OF) tests. In males, anxiety-like behaviors were determined prior to and after DIO (60% high fat, 8 weeks). In females, the estrous cycle was monitored daily for 12 days prior to behavioral testing in the EPM without diet manipulation. For all studies food intake and weight were monitored throughout. We found that, as expected, OP rats gained substantially more weight and fat mass than OR rats when maintained on standard lab chow; this was further exacerbated by high fat diet (HF). Anxiety-like behaviors in the EPM and OF tests were enhanced in obese male and female OP rats. Importantly, differences in anxiety were not present prior to obesity in male OP rats, and the magnitude of anxiety-like behavior was positively correlated with weight gain. In males, consumption of a 60% HF produced obesity in both OP and OR rats. However, DIO in OR male rats was not sufficient to enhance anxiety-like behaviors. In ongoing studies we are determining the effect of HF-DIO in anxiety-like behaviors in females and the effect of the estrous cycle in these behaviors in these models. Our studies to date suggest that increases in anxiety emerge along with obesity in OP rats and that interaction between predisposition and weight gain contribute to this behavioral difference.
Loss but not reduction of LRRK1 results in motor deficits in mice.

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Parkinson’s disease (PD) is one of the most common age-related neurodegenerative motor disorders, neuropathologically characterized by a loss of dopamine neurons in the substantia nigra. The greatest genetic contributor to PD is leucine rich repeat kinase 2 (LRRK2). Dominant missense mutations in LRRK2 are the most common cause of inherited PD and patients carrying mutations in LRRK2 show clinical symptoms indistinguishable from typical sporadic PD. However the function of LRRK2 is unclear, because LRRK2 knockout (KO) mice do not exhibit a PD behavior or other neuropathological phenotype. These data contrast with the robust phenotypes seen in drosophila or c. elegans following the deletion of the LRRK2 homologue in these organisms. However, drosophila and c. elegans contain only one LRRK gene while mice and humans contain two (LRRK2 and LRRK1). We hypothesize that LRRK1 may compensate for LRRK2, and that removing both genes may reveal motor deficits. To test this, we first had to create a LRRK1 mouse. We did this by using a gene targeting technique that knocked in a lacZ reporter followed by a STOP cassette into the endogenous LRRK1 gene, creating a null allele. LRRK1 knockout animals were generated then crossed with established LRRK2 knockout animals to create a LRRK double knockout. However, this strategy only reduced LRRK1 mRNA levels, and did not completely remove LRRK1. This reduction did not result in any motor deficits or neuronal deficits. Since this LRRK1 knockout mouse did not have LRRK1 completely removed, we took these mice and crossed them to mice expressing cre-recombinase in all tissues. Exons 4 and 5 were flanked by flxp sites, and were excised resulting in the global deletion of LRRK1. With this new model, fewer LRRK1 knockout homozygous pups were born than expected by Mendelian ratios. Pups that did survive weigh less than their wild type and heterozygous litter mates. LRRK1 knockout mice showed motor deficits in the open field and grip strength tests compared to their wild type littermates. In conclusion, low levels of LRRK1 are sufficient to compensate for LRRK2; however complete removal LRRK1 results in motor deficits that are seen in PD suggesting that LRRK1 may play a role in PD.
The protein kinase Cβ inhibitor, enzastaurin, decreases amphetamine-mediated behaviors in rats
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Amphetamines elicit their motivating effects by increasing extracellular dopamine levels in the brain. Extracellular dopamine levels are regulated by the dopamine transporter (DAT), a transmembrane protein that transports dopamine from the synapse into the cell. Amphetamines are taken up by DAT and reverse the transporter to release dopamine into the synapse. One protein that has been shown to be important for the mechanism of amphetamine's action is protein kinase Cβ (PKCβ). The inhibition of PKCβ reduces amphetamine-stimulated dopamine efflux through DAT in vitro and in vivo while having no effect on dopamine uptake. While the effects of PKC inhibitors on amphetamine-stimulated dopamine efflux have been characterized, it is important to understand the effects of PKCβ inhibition on amphetamine-stimulated behaviors. The goal of this study was to determine whether inhibition of PKCβ will decrease amphetamine-stimulated locomotion and amphetamine self-administration. For all experiments, the selective PKCβ inhibitor enzastaurin was administered to male Sprague-Dawley rats by intracerebroventricular injections 3, 6, or 18 hours prior to evaluating amphetamine-mediated behaviors. Locomotor activity was measured in infrared beam break boxes following administration of a single dose (0.32-3.2 mg/kg) of amphetamine. In self-administration studies under a fixed ratio 5 (FR5) schedule of reinforcement, rats earned infusions of amphetamine (0.032 mg/kg/infusion) or sucrose pellets in daily sessions. Pretreatment times less than 18 hours were insufficient to decrease amphetamine-stimulated locomotor activity. An 18-hour pretreatment with 10 pmol enzastaurin reduced amphetamine-stimulated locomotion at lower amphetamine doses, but this effect was surmounted by larger doses (3.2 mg/kg) of amphetamine. In amphetamine self-administration studies, an 18-hour pretreatment of 10 pmol enzastaurin decreased the number of amphetamine infusions by over 60%, but a 3-hour pretreatment with enzastaurin had no effect. This effect was specific for amphetamine because enzastaurin did not alter the number of sucrose pellets earned. This study demonstrated that PKCβ inhibition attenuated amphetamine mediated behaviors, likely in a surmountable fashion. PKCβ inhibitors may be useful therapeutic targets for decreasing amphetamine use and abuse without altering natural rewards. Future work will investigate the mechanisms by which PKCβ inhibition alters amphetamine-stimulated behaviors. Funded by DA11697 and T32-GM007767.
Mu-opioid receptor (MOR) agonists have long been used in the treatment of pain and are currently the standard for pain management in the clinic. However, their use produces many adverse effects, such as the development of tolerance, physical dependence, euphoria, and constipation. The co-administration of a MOR agonist with a delta opioid receptor (DOR) antagonist may produce MOR-mediated analgesia with reduced side effects. However, administering drug cocktails has considerable complications related to potential diverse pharmacokinetic properties of the two chemical entities. We therefore explored the development of multifunctional ligands that display MOR agonism and DOR antagonism in a single molecule. We have previously reported a cyclic peptide, VRP26, which displays the desired MOR agonist/DOR antagonist profile in vitro and produces opioid mediated antinociception in male C57BL/6N mice. The goals of the current study were to evaluate whether VRP26: 1) crosses the blood brain barrier and 2) produces tolerance, dependence, rewarding effects, or constipation. The effects of VRP26 on centrally-mediated behaviors in the tail suspension test were evaluated. Tolerance and dependence were evaluated following continuous VRP26 infusion for 7 days, rewarding effects were evaluated in a conditioned place preference assay, and constipation was evaluated by fecal bolus production after acute drug administration. VRP26 attenuated antidepressant-like effects induced by the DOR agonist SNC80. Unlike fentanyl, 7 day continuous administration of VRP26 failed to produce a rightward shift in the antinociceptive dose response curve and produced fewer signs of naltrexone-precipitated withdrawal than fentanyl. VRP26, unlike fentanyl, did not produce significant conditioned place preference. VRP26 was less potent that morphine in producing constipating effects. In conclusion, VRP26 produces centrally-mediated antinociception, with limited development of tolerance, dependence, or rewarding effects. Further, VRP26 has a greater therapeutic index between antinociceptive and constipating effects, demonstrating potential increased tolerability. VRP26 demonstrates proof of concept that mixed efficacy opioid ligands may be better alternatives to traditional opioid analgesics for chronic pain management, producing pain relief with limited negative effects.
Effects of repeated 3,4-methylenedioxymethamphetamine (MDMA) administration on behavioral assessments of anxiety in adult male Sprague-Dawley rats.


3,4-Methylenedioxymethamphetamine (MDMA) is a popular recreational drug of abuse that has shown promise in clinical trials for the treatment of post-traumatic stress disorder. Excessive recreational MDMA abuse has been linked to several adverse psychological effects, including anxiety, sleep disturbances, and attention deficits. In preclinical investigations, repeated MDMA exposure (10mg/kg for 10 days) during early and late adolescent periods produced persistent increases in anxiety-related behaviors when rats were later assessed as adults. The aim of the current study was to determine if repeated MDMA administration in older adult rats produced comparable anxiogenic effects. Thirty adult male Sprague-Dawley rats were randomly assigned to one of three treatment groups that received once-daily injections of saline, 5 mg/kg MDMA, or 10 mg/kg MDMA for 10 days. Immediately following a 10-day drug washout period, rats were assessed in three standard assessments of anxiety, including a light-dark box, open-field test, and elevated-plus maze over a period of three days. Statistical analyses revealed no significant differences among treatment groups on the majority of dependent measures. These results are inconsistent with previous findings following similar drug exposure in younger rats, suggesting older animals may display more resilience to the potentially adverse effects of MDMA. However, a direct comparison to the aforementioned previous study is not possible, due to the shorter drug washout period used in the present study. As such, a 10 day drug washout period may have been an insufficient incubation period to establish anxiogenesis. Moreover, the lack of statistical significance in the current study may be attributed to the high variability among test subjects, which could be perhaps reduced by pre-screening animals for anxiety-related behaviors prior to drug administration. In consideration of ongoing clinical initiatives to establish MDMA as an adjuvant treatment for PTSD, further preclinical investigations in adult animals may be warranted.
Approximately 86% of Americans currently suffer from a gambling addiction. Young adults ages 18-24, the typical age of college students, are at a higher risk than any other population to develop a gambling addiction. To gain further insight into the effects various audio cues have on individuals exhibiting risky behavior, college students (n=22) participated in a variation of the Iowa Gambling Task (IGT) while listening to positive, negative, or no cues. Participants' affective state was hypothesized to be more positive if exposed to positive cues and more negative if exposed to negative cues regardless of whether the participant "wins" or "loses" which would result in the gambler's propensity to continue engaging in risky behavior. Additionally, participants who received the negative auditory cues would display elevated heart rates.

Participants were recruited from a small liberal arts college. They were given an affective state questionnaire, and their pulse, blood pressure, and skin conductance response were measured. Each participant was given 100 points with which to gamble. Participants were given headphones so they could listen to the negative or auditory cues before beginning the IGT. Two decks of twelve playing cards each were placed in front of the participant to draw one at a time. Playing cards had either a negative or positive value indicated on the card. The two decks of cards were sorted into a more risky deck yielding high losses and wins, and a conservative deck yielding lower losses and wins.

This study showed an increase in the heart rate and diastolic blood pressure levels of those who received positive cues and won was higher in the post-game assessment compared to the baseline assessment. Qualitative data suggest that those receiving positive cues were more likely to play again than those who received the negative cues or no cues. Only 12.5% of participants who received positive cues won the IGT which suggest similar probability of a gambler winning in a casino-like setting.

From the study presented, results suggest positive auditory cues may increase a participant's propensity to continue engaging in risky behavior, and this increased propensity was associated with an elevated physiological response. The authors would like to thank Rochester College for providing the equipment and facilities for this study.
Migration of BrdU Positive Cells in Adult Mammalian Retina after Treatment with an α7 nAChR Agonist

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Proliferation and regeneration of adult mammalian neurons does not typically occur. However, previous studies from this lab have demonstrated that eye drop application of the alpha7 nicotinic acetylcholine receptor agonist (α7 nAChR), PNU-282987, increased the number of retinal ganglion cells (RGCs) compared to controlled untreated conditions in adult rats. We investigated the cause of this increase in retinal neurons after treatment with the α7 nAChR agonist. Specifically, we examined whether PNU-282987 triggered mitosis in the adult retina. Towards this end, the right eye of adult Long Evans rats were treated with eye drops containing various concentrations of PNU-282987 in PBS containing 1 mg/ml bromodeoxyuridine (BrdU). BrdU labels mitotically active cells as well as cells undergoing unscheduled DNA synthesis. The left eye was untreated and acted as an internal control. Once eye drop treatments were completed, animals were sacrificed, retinas were removed, immunostained with an antibody agonist BrdU or proliferating cell nuclear antigen (PCNA) to label mitotically active cells, anti-vimentin to label Müller glia and DAPI to label cell nuclei. After immunocytochemical processing, retinas were sectioned, mounted and viewed under a Nikon confocal microscope. Our results demonstrated BrdU positive cells in different layers of the retina at different time points. Studies were then performed to examine the origin and migration of these cells throughout the retina. Results provided evidence that BrdU positive cells originate from Müller glia at 36 hours following eye drop application and then migrate through the retina to the photoreceptor layer (ONL) and ganglion cell layer (GCL). PCNA positive cells were found in the retina after PNU-282987 treatment as early as 12 hours after eye drop application and arise before any BrdU positive cells. These results support the hypothesis that an α7 nAChR agonist can induce proliferation of adult mammalian neurons. Proliferation of new retinal neurons can lead to the reverse of vision loss associated with neuron degenerative disease, age related effects, and reversing traumatic injuries. This work was supported by an NIH NEI grant (EY 022795) issued to Dr. Linn.
BACLOFEN-YOHIMBINE INTERACTION IN MALE AND FEMALE RATS WITH HIGH LEVELS OF ETHANOL SELF-ADMINISTRATION

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Alcohol is said to cause more overall harm than any other drug. The cessation of alcohol consumption can be a stressful process for alcoholics. Stress has shown to increase alcohol consumption, and induce relapse in recovering alcoholics. Baclofen may be useful as a potential treatment option for alcohol dependence, particularly for individuals with comorbid stress disorders. Yohimbine, a presynaptic alpha-2 adrenoreceptor antagonist, increases adrenergic activity and is considered a pharmacological stressor. Pharmacotherapies that reduce stress, such as that caused by yohimbine, may decrease alcohol consumption in alcoholics.

The purpose of the present study was to examine the interaction of baclofen and yohimbine in a rat model of alcohol self-administration with high levels of responding and drug intake in both males and females. Rats were trained in operant chambers to respond for ethanol. Ethanol intake and responding was measured during daily 30 minute sessions. Once high levels of responding and intake were established as baseline, rats were pretreated with injections of yohimbine (1.25 mg/kg), baclofen (0.3 mg/kg, 1 mg/kg or 3 mg/kg), or baclofen in combination with yohimbine. As expected, injections with yohimbine systematically increased responding and intake. Baclofen alone suppressed self-administration at the highest dose (3 mg/kg) and it continued to suppress self-administration when given in conjunction with yohimbine. Smaller doses of baclofen failed to inhibit yohimbine’s ability to increase ethanol self-administration. Although responding was similar between male and female rats, the female rats had greater ethanol intakes due to differences in weight. The pattern of interaction for baclofen and yohimbine was similar in both males and females. In conclusion, baclofen’s ability to alter alcohol self-administration in the presence of stress may depend upon the type of alcohol solution (sweetened vs. unsweetened), overall rate of responding, injection order, and other variables.
CAN CRAYFISH RECOVER CHEMOSENSORY ABILITIES FOLLOWING AN ACUTE ATRAZINE EXPOSURE?

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Atrazine is one of the most heavily applied herbicides in the United States. Following spring run-off it can often be found in concentrations of >100 ppb in Midwest streams and rivers, exposing aquatic organisms to elevated concentrations for up to three weeks. We have previously shown that an acute exposure to an environmentally relevant concentration of atrazine causes deleterious effects in the chemosensory responses of crayfish to both food odors and mate odors. Additionally, crayfish could not recover these chemosensory responses following a 72-hour recovery period in clean water. Crayfish are nocturnally active and often found in turbid environments. Given this, they rely extensively on waterborne odors for locating mates, food and shelters as well as to avoid predators. In this study, crayfish (Orconectes virilis) were exposed to atrazine (80 ppb for 96 hours) and recovery was examined over a 15-day period. After treating the crayfish, a Y-maze was used and fish-flavored gelatin was placed in one arm while control gelatin was placed in the other. Time spent in the food arm of the Y-maze, time at the odor source, walking speed and the total amount of food consumed during the 15-day recovery period were examined every three days. Upon analysis, data indicates some recovery is possible after approximately nine days in fresh water. Results from this experiment are important because crayfish are polytrophic, meaning they feed on and become prey for all levels of the aquatic food web. Additionally, crayfish facilitate the transfer of energy between benthic and terrestrial food webs. Since crayfish rely heavily on their chemosensory abilities to acquire food, the long-term impacts of atrazine exposure could affect population size in areas where atrazine is heavily applied. Any reduction in crayfish population size will, in turn, affect aquatic food web dynamics.
The Temporal Regulation of Optic Nerve and Photoreceptor Regeneration

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Planarians are a powerful neuroregeneration model with the unique ability to regrow their entire central nervous system, including the brain, optic nerves and photoreceptor cells. However, the mechanisms by which they can regenerate neural tissues are only imperfectly understood. One complication to parsing these mechanisms is that tissue regeneration is typically studied in the laboratory setting using standard injury models. However in nature injuries vary greatly in both the amount and location of tissues lost, and it is not clear how these variables affect either the timing or ability of neural tissues to regenerate. Planarians have the unique ability to regenerate from many different injury types, allowing us to study the effects of different injuries on regeneration timelines. We followed the timing of regeneration for one organ, the eye, after multiple injury types (single and double eye ablation, and decapitation) in Schmidtea mediterranea planaria. Our data reveal that the timing of regeneration remained constant despite changing injury parameters. Optic tissue regrowth, nerve re-innervation, and functional recovery were not statistically different between injury types (even when the animal was simultaneously regrowing its brain). Changes in metabolic rate (i.e. starving versus fed regenerates) also had no effect on regeneration timelines. In addition, our data suggest there may exist a role for optic nerve degeneration following eye ablation, prior to optic nerve and photoreceptor regeneration. Together, our results suggest that the temporal regulation of planarian eye regeneration (and thus of eye stem cell differentiation) is tightly controlled and resistant to variations in injury type.
Fragile X Syndrome (FXS) is the leading genetic form of autism, caused by transcriptional silencing of the fragile X mental retardation 1 (FMR1) gene. The phenotype associated with FXS is multifaceted and diverse, indicative of the complex functional role of Fragile X mental retardation protein (FMRP) within neural systems. Recent studies have demonstrated visual impairment in FXS patients; however underlying mechanisms remain unknown. The dorsal lateral geniculate nucleus (dLGN) plays a dynamic, obligatory role in the transfer of primary visual information from the retina to primary visual neocortex. The present study is aimed to uncover possible alterations in inhibitory neurotransmission in the dLGN associated with Fmr1 knockout (KO) mice utilizing electrophysiological and molecular approaches. We found significant decrease in metabotropic glutamate receptor (mGluR)-dependent modulation of GABA_A receptor-mediated inhibition at retinogeniculate synapses. Inhibitory activity elicited by both pharmacological and synaptic activation of mGluR_5, a specific subtype of group I mGluRs, was significantly reduced in thalamocortical neurons of Fmr1 KO mice. Consistent with this observation, there was significant reduction in the mGluR5, but not mGluR1, protein levels in dLGN of Fmr1 KO mice. Our data provide possible mechanistic links between synaptic alterations and visual deficits associated with FXS.
Evaluation of the discriminable stimulus effects of 0.3 mg/kg 3,4-methylenedioxyamphetamine (MDPV) and 1.0 mg/kg 4-methylmethcathinone (4-MMC) using a drug discrimination procedure in male Sprague-Dawley rats

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Previous studies using intravenous self-administration procedures in non-human experimental subjects have revealed that the synthetic cathinones, 3,4-methylenedioxyamphetamine (MDPV) and 4-methylcathinone (4-MMC, mephedrone), can function as reinforcers with effects comparable to prototypical drugs of abuse. These findings suggest these drugs possess high abuse potential and presumably elicit interoceptive effects in experimental subjects. Relatively fewer studies have investigated the discriminable stimulus effects of synthetic cathinones using drug discrimination procedures. Further research using drug discrimination procedures will broaden understanding of the interoceptive effects produced by MDPV and 4-MMC. The present experiment investigated the discriminable stimulus effects of 0.3 mg/kg MDPV (N=8) or 1.0 mg/kg 4-MMC (N=8) using a two-lever drug discrimination procedure in male Sprague-Dawley rats. The MDPV- and 4-MMC-trained rats displayed no difference in the number of sessions required to reach the criterion for the acquisition of the discrimination. Stimulus substitution tests were conducted with the following compounds: MDPV (0.01-1.0 mg/kg), 4-MMC (0.01-3.0), d-amphetamine (0.03-1.0), methamphetamine (0.03-1.0), cocaine (0.03-10.0), 3,4-methylenedioxymethamphetamine (0.1-3.0), lysergic acid diethylamide (0.01-0.1), and fenfluramine (0.1-3.0). Results revealed that MDPV, d-amphetamine, methamphetamine, and cocaine produced full-substitution in 0.3 mg/kg MDPV-trained rats, and 4-MMC, MDPV, d-amphetamine, methamphetamine, cocaine, and 3,4-methylenedioxymethamphetamine produced full-substitution in 1.0 mg/kg 4-MMC-trained rats. This study is currently in progress and full results will be forthcoming. This experiment will expand upon the current behavioral profile of MDPV and 4-MMC and may further assist in characterizing their subjective effects in habitual users.
Planarian Photophobic Behavior is Mediated by Both Ocular and Dermal Phototransduction

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Planarians, a popular regeneration model organism, are free-living aquatic flatworms that possess strong photophobic responses to light. Although their prototypic eye is comprised of only two cell types, pigment cells and photoreceptor neurons, planarian phototransduction is surprisingly complex. Like many aquatic animals, planarians possess both ocular (via the eye) and dermal phototransduction (via the skin). However, their behavioral responses to light have mainly been studied in terms of ocular phototransduction, while relatively little is known about the mechanisms regulating their dermal responses to light. We sought to increase our understanding of planarian phototransduction by investigating 1) the wavelengths involved in ocular phototactic responses and 2) the mechanisms that enable dermal phototransduction in these animals. Planarian photophobia is traditionally assessed in behavioral studies by their (ocular) avoidance to white light, which is comprised of multiple wavelengths. However, many animals display different behavioral responses to specific wavelengths, suggesting that white light may mask important differences. Our data show that planarians possess a hierarchy of behavioral responses across a range of wavelengths, including ultraviolet and infrared light (which had not previously been examined). Responses were directly correlated with wavelength, with the shortest wavelengths (near ultraviolet) producing the most intense photophobic responses and longer wavelengths causing no effect (red) or an opposite effect (infrared). We also discovered that photophobic behavior is comprised of two different response types: a general response that occurs due to changes in luminosity and a wavelength-specific response that involves the different behaviors correlated with individual wavelengths. Next we characterized the planarian ability to detect light using mechanisms outside of the eye, a phenomena which has been reported in the literature but not molecularly analyzed. Several species, including C. elegans, leeches, and Drosophila larvae, use non-ocular phototransduction for feeding and avoidance behavior. We found that when either eyeless or decapitated worms are exposed to ultraviolet light, intense photophobic behaviors are still observed. Furthermore, our data revealed that RNAi to the transient receptor potential ion channel (TRPA1) results in worms that lack dermal photophobic responses to ultraviolet light, suggesting that TRPA1 is required for dermal phototransduction. Together, our results illustrate the complexity and sophistication of the planarian visual system. This knowledge will be of great use for both dissecting planarian eye function and investigating the mechanisms by which they can regenerate their eyes.
EXPLORING THE PATTERN OF GENE EXPRESSION ACROSS THE HUMAN AMYGDALA: CHARACTERIZING CIRCUITS THAT ARE VULNERABLE TO MAJOR DEPRESSIVE DISORDER

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Major Depressive Disorder (MDD) is a common mood disorder that affects millions of people worldwide. In our research we seek to narrow down which factors may be the cause of the disorder by observing the importance of the amygdala for MDD. The amygdala plays a number of roles within the brain, due to its myriad connections, but perhaps the most notable function is its involvement in emotion. In order to better understand the molecular characteristics of the different regions of the amygdala, sub-regions of the amygdala from human control subject brains were identified and cryo-sectioned. The gene expression of these sub-regions was then obtained through the use of laser capture microscopy and microarray. This data was transformed into large gene expression matrices and analyzed through the use of R programming. A cross comparison was then done with data obtained from the Allen Brain Atlas, a large online database containing anatomical and genomic maps of mouse and human brains. Through R coding, gene expression across the targeted amygdala sub-regions was compared between each dataset. We found that in general the regions we expected to show strong correlations remained relatively consistent between datasets. The correlations across the two different datasets were predominantly much less pronounced than within the same dataset, which is to be expected due to differences in microarray, dissection, as well as normalization processes. Overall our comparisons validated the utility of both gene expression datasets for understanding the amygdala, and provide a useful tool with which to analyze amygdala sub-regions between each other and other brain regions. The findings from this project offer valuable insight into the role of the amygdala and the various functions of each amygdalar sub-region. This research can then be used to further studies in MDD by providing a framework of amygdala gene expression in healthy controls.
An Overview of An Improved Experimental Design: Developmental Aromatase Inhibition and Endocrine Disruption and the Potential Effects on Organization of Brain Morphology in the Norway Rat (*Rattus norvegicus*)

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In classic work by William C. Young’s research laboratory, it was established that the organization of mammalian brain morphology is guided by expressed gonadal hormones *in utero*. Furthermore, it has been established that specific enzymatic alteration of these hormones can occur in the undifferentiated neuron. Therefore, the genetic sex of an organism may typically drives phenotypic development of sexual morphology and the brain.

Experiments involving manipulation of embryonic environments in *oviparous* avian and fish models has demonstrated it is possible for phenotypic sex expression to occur that is opposite that of genotype. This opposite phenotypic expression is possible both in body morphology and in brain organization. However, there are currently no *viviparous* organisms where induced phenotypic sex opposite of genotype has been demonstrated, most likely due to chemical complexities associated with internal gestation.

Here, we theorize a chemical cocktail, specifically use of an aromatase inhibitor plus specific endocrine disrupting compounds, may be able to influence early development by reshaping the mammalian intrauterine environment enough to allow phenotypic expression of sexual morphology opposite that of genotype in mammals. In this poster, we present a detailed look at our experimental design in which we are striving to induce phenotypic sex development opposite of genotype in the Norway rat. In our proposed research model, sexually indifferent morphology is maintained through gestational day 10 following fertilization. Our chemical mixture will be introduced to our subjects beginning within this undifferentiated developmental stage of sexual organization and continued through parturition. Administration of the chemical mixture to the intrauterine environment will impact gestation in ways we suspect will alter development resulting in changes in performance on a variety of post-natal behavioral and morphological tests relevant to sex differentiation, including brain organization, especially of the SDN (sexually dimorphic nucleus). By comparing control and treatment populations at the neural, morphological, reproductive, and behavioral levels, we hope to gain deeper insight and understanding of the mechanisms driving sexual differentiation in mammals.
Reduced voluntary ethanol intake and preference following genetic deletion or pharmacological inhibition of adenylyl cyclase 1

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Intermittent, voluntary access to ethanol produces a gradual escalation to excessive consumption over several weeks, which mirrors many key features of clinical alcohol abuse. Ethanol consumption in this model increases GluN2B phosphorylation and persistently upregulates GluN2B-containing NMDAR transmission selectively in the dorsal medial striatum. As we demonstrate that adenylyl cyclase 1 (AC1) signaling is crucial for ethanol-induced striatal GluN2B phosphorylation, the impact of loss of AC1 activity on intermittent ethanol consumption was assessed. Single-housed control (WT, C57BL/6) or AC1 knock-out (AC1KO) mice had 24 h concurrent access to one bottle containing 20% ethanol and one bottle containing tap water (days 1, 3, 5), followed with access to two bottles containing tap water (days 2, 4, 6 and 7). AC1KO mice exhibited significantly reduced ethanol intake and preference compared to WT mice throughout the 13 ethanol access sessions. To mechanistically validate the involvement of AC1 in this suppression effect, a selective pharmacological AC1 inhibitor NB001 was used. In ethanol-naïve WT mice, NB001 pre-treatment (10 mg/kg, i.p.) 30 min prior to ethanol bottle presentation significantly suppressed ethanol intake and preference compared to saline-treated controls over 5 ethanol access sessions. These data support the viability of a targeted AC1 intervention to attenuate excessive ethanol intake and extend previous evidence that calcium-stimulated ACs contribute to ethanol behavioral responses. Furthermore, these data suggest that NB001 demonstrates utility as a potential therapeutic for use in alcohol addiction.

This work was supported with resources at the John D. Dingell VA Medical Center (Detroit, MI) and funds from the Wayne State University Department of Neurosurgery (A.C.C.).
In the United States 1.7 million people receive a TBI annually and it is estimated there are 5.3 million people in the U.S. living with long term disabilities. Currently, there are no clinical treatments that are effective in alleviating the functional deficits of TBI. Progesterone (PROG), a neurosteroid with pleiotropic effects has been shown to be beneficial in multiple brain injury models. The purpose of this study was to investigate the neurologically protective effect of progesterone following traumatic brain injury in animals reared in Enriched Environments (EE). The current study used 27 male Long-Evans rats purchased at post-natal day 25 and reared to maturity in EE. After 91 days, 18 subjects received a bilateral controlled cortical impact (CCI) placed over the medial frontal cortex to produce a moderately severe injury. Rats were administered intraperitoneal injection of either 10mg/kg PROG or vehicle injections (peanut oil) 4h post-injury and every 12h for 72h following the initial injection. Seven days post-injury the rats were tested on several behavioral tasks including the open field test, Barnes maze, Morris water maze, rotor-rod task, elevated plus maze, and forced swim task. Results from behavioral testing suggest that overall the intact animals performed significantly better than injured animals. Contrary to the established literature we found an intermediate effect of PROG when animals were tested on the MWM and rotor rod tasks, where intact animals performed significantly better than untreated group. An interesting additional finding using Elevated plus maze (EPM) to assess anxiety-like behaviors, overall intact animals spent significantly greater time in the closed arms than the injured groups. Analysis of the behavioral data from this study has shown that enriched housing before injury can impact behavior tasks in intact animals as well as functional recovery in injured animals when in combination with PROG. Future research should explore potential mechanisms related to pre-enriched housing that may influence recovery from TBI.
Interference in a bimanual task is not reflected in intracortical inhibition

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Recent studies have shown that motor repetition practice and motor adaptation tasks have differential effects on intrahemispheric inhibition of the motor cortex. Simple repetition was shown to decrease inhibition in the motor cortex, whereas motor adaptation tasks did not appear to affect inhibition. Little is known about intracortical inhibition in the context of bimanual movements that elicit interference in one hand. Our task required participants to adapt to a visuomotor perturbation in one hand, while moving the other hand without visual feedback. Previous work has found differential interference patterns in participants performing this task. The goal of our study was to compare inhibition in both hemispheres before and after a bimanual movement task, using transcranial magnetic stimulation (TMS), and measuring short-interval intracortical inhibition (SICI).

Thirty right-handed participants (mean age: 23.6 yrs.) performed a bimanual center-out task, moving two KINARM end-point robots from two home positions (one per hand) to peripheral targets at 90° or 270° (distance: 10cm); vision of the hands was occluded. Participants performed a visual baseline condition, with both cursors visible, and a kinesthetic baseline (pre-exposure) where visual feedback was only present for the right hand, but not the left. Twenty participants were then exposed to 120 trials with a 40° rotation of visual feedback in the right hand (exposure), and were instructed to continue moving straight with the ‘invisible’ left hand. After this, the baseline condition was reintroduced in the right hand to assess aftereffects of the adaptation. Control participants received veridical visual feedback for the right hand throughout the experiment. Initial directional error (IDE), measured at peak velocity, was used to determine the feed-forward directional control component of the movements as a measure of adaptation.

For TMS, active motor thresholds for the extensor digitorum were determined over each hemisphere and recorded with surface electromyography (EMG). Cortical excitability and inhibition were determined prior to the adaptation task, using a single pulse paradigm to elicit a 1mV peak-to-peak amplitude motor evoked potential (MEP), and a paired-pulse paradigm for SICI at 2.5ms interstimulus interval. Inhibition was calculated as a percentage of the single pulse MEP (1mV) amplitude. MEPs were obtained again at the same stimulator output immediately after exposure.

The behavioral results showed a reduction of IDE over the exposure trials, indicating that in all ‘rotated’ participants the visible hand adapted to the kinematic perturbation, whereas the invisible left hand showed small, yet consistent directional interference effects. Fourteen of the rotated participants showed interference in the same direction as the perturbed right hand (ISO), and six showed interference in the opposite direction (ANISO). TMS results showed, in the hemisphere controlling the visible hand, there was a release of inhibition following the movement task for the control, but not the adapting groups (p < 0.05). In the hemisphere controlling the invisible hand, neither the control nor the adapting groups showed a change in intracortical inhibition. The results suggest that different interference characteristics do not seem to be mediated through intracortical inhibition.
Assessment of behavioral sensitization following repeated exposure to low dose mixtures of 4-methylmethcathinone (4-MMC) and 3,4-methylenedioxymethamphetamine (MDMA) in male Sprague-Dawley rats

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Recreational abuse of synthetic cathinones is a significant public health concern. Although the Drug Enforcement Administration placed several of the most common constituents of illicit “bath salts” permanently on schedule 1, their use is still prevalent. In humans, the concomitant use of cathinone derivatives and other drugs is commonly reported, especially with other psychostimulant drugs, such as cocaine, methamphetamine, and 3,4-methylenedioxymethamphetamine (MDMA). However, there is currently a paucity of scientific research regarding the behavioral and neurochemical effects of psychostimulant drug mixtures. The behavioral sensitization paradigm is a preclinical tool that can be used to assess the influence of prior drug exposure on sensitivity to the behavioral effects of other drugs. Utilizing this paradigm, the present study assessed the locomotor stimulant effects of MDMA and 4-methylmethcathinone (4-MMC), administered individually and in mixtures. Male Sprague-Dawley rats (N=120) were randomly assigned to one of 15 treatment groups (N=8) and administered intraperitoneal injections of one of the following treatments once per day for seven consecutive days: saline, 3 mg/kg MDMA, 4-MMC (1 or 5 mg/kg), 3 mg/kg MDMA+4-MMC (1.0 or 5.0 mg/kg). A 10 day drug-free incubation period followed, after which rats were challenged with a single I.P. injection of either saline, 4-MMC (1.0 or 5.0 mg/kg), or 3 mg/kg MDMA. Some groups of animals received the same drug they received during the first seven days, while other groups received the other drug. On days 1 and 7, and on the post-incubation challenge day, locomotor activity was monitored for one hour immediately before and one hour immediately after injections. Results indicated the induction of sensitization by MDMA. While neither dose of 4-MMC induced behavioral sensitization, the induction of sensitization was greater with mixtures of 4-MMC and MDMA compared to that produced by MDMA alone. Furthermore, the expression of sensitization following the 10 day washout was evident only in those animals that were previously exposed to MDMA or 5 mg/kg 4-MMC + MDMA. These findings suggest the possibility of increased abuse liability of 4-MMC when used concomitantly with MDMA or following prior MDMA use. Further investigations utilizing behavioral indices of abuse liability (e.g., place conditioning, drug self-administration) to assess the combined effects of MDMA and 4-MMC may be warranted.
DECREASED SEIZURE THRESHOLD IN MICE FOLLOWING TWENTY-FOUR HOUR EXPOSURE TO TOLUENE VAPOR S.P. Callan, C.J. Davidson, & S.E. Bowen Department of Psychology, Wayne State University, Detroit, MI 48201, U.S.A.

The intentional misuse of volatile solvents is a persistent public health concern. Limited self-report data suggests that chronic inhalant abusers experience withdrawal symptoms including anxiety and seizure symptoms. However, these symptoms have never been explored in a preclinical model and are not considered part of the DSM-V criteria for an Inhalant Use Disorder. For this experiment, 76 young adult male Swiss Webster mice were exposed to either 5,000 ppm toluene vapor or air (0 ppm) for 24 consecutive hours beginning on postnatal day (PND) 30. Following the 24 hour exposure, mice were allowed to recover for 3 hours before behavioral testing began. In the 1st experiment, mice were tested for handling-induced seizure activity every hour for 6 hours (and again at 24 hours). As compared to controls, toluene-abstinent animals showed persistent clonic seizure activity throughout the 6 hour period. In the 2nd experiment, mice were given a single i.p. injection of pentylentetrazole (PTZ; 42 or 48 mg/kg) to induce seizure activity. Mice were observed for 30 min and seizure activity was scored for severity using criteria adapted from the Functional Observational Battery. As compared to air controls, toluene-abstinent mice displayed a significant increase in seizure symptoms. In the 3rd experiment, previously exposed toluene mice were re-exposed to toluene vapor for 30 min following the three hour abstinence period. Following toluene re-exposure, these mice were tested for seizure severity with 42 or 48 mg/kg PTZ. Toluene re-exposure significantly reduced the severity of the seizure response. Taken together, these results suggest that toluene abstinence lowers seizure threshold in mice and that toluene re-exposure raises it. These findings provide support for clinical reports of a physical withdrawal syndrome from inhalants, which has implications for the successful diagnosis and subsequent treatment of Inhalant Use Disorders.

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Antidepressant-like effects of the delta opioid receptor agonist SNC80 in Sprague-Dawley and Wistar-Kyoto rat strains

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Depression is one of the most common mental disorders in the United States. Currently, the most frequently prescribed antidepressant drugs are selective serotonin reuptake inhibitors (SSRIs). However, SSRIs are not effective in a wide range of individuals or if they are effective, there is generally a therapeutic lag of up to six weeks before a measurable therapeutic response is observed. Therefore, novel pharmacotherapies for depression need to be investigated. To determine the potential of delta opioid receptor (DOPr) agonists as effective antidepressants in an animal model of depression, we evaluated the effects of the DOPr agonist SNC80 in Sprague-Dawley (SD) and Wistar-Kyoto (WKY) rats in the forced swim test (FST). The WKY rat strain is considered to have a behavioral phenotype consistent with symptoms of depression and is often used to evaluate antidepressant-like effects of novel drugs. To investigate whether underlying changes in the DOPr system contribute to the depressive-like phenotype of WKY rats, DOPr receptor number and function were evaluated using [3H]DPDPE saturation binding assays and agonist-stimulated [35S]GTPγS autoradiography, respectively. Endogenous enkephalin (ENK) levels were also measured by in vivo microdialysis. SNC80 produced antidepressant-like effects in both SD and WKY rats in the FST; however, SNC80 was more potent and efficacious in SD rats as compared with WKY rats. Although DOPr expression levels and endogenous ENK levels were similar in both rat strains, there was greater SNC80 stimulated G protein activation in certain brain regions of SD rats as compared with WKY rats. Our current findings suggest that DOPr agonists may be useful as antidepressants and that the depressive-like phenotypes of WKY rats may be due to decreased DOPr system activity. These studies further support the role of the DOPr system in regulating mood and emotion and the DOPr as a novel target for treating depression.
Studies have shown that combat veterans with lifetime PTSD also met the criteria for alcohol disorder. Chronic alcohol consumption and withdrawal cause anxiety, increased alcohol consumption behavior, and alcohol dependence, which may complicate PTSD treatment. Research has shown that the endocannabinoid system is involved in both alcohol dependence and fear processing, and that dopamine-2 receptor (D2) is involved in endocannabinoid-mediated depression of corticostriatal glutamate release, however, the mechanism by which they interact are unclear. Therefore, the effects of traumatic stress combined with chronic ethanol exposure on cannabinoid 1 (CB1) and D2 receptors were examined. Male C57BL/6 mice were exposed to mouse Single Prolonged Stress (mSPS), an animal model that shows good validity in producing PTSD-like behavior. Seven days after mSPS exposure, mice were placed in inhalation chambers and received chronic intermittent ethanol vapor (CIEv) or air exposure for 16 hr/day, 4 days/cycle for 4 cycles. Mice were killed 72 hours after the last vapor or air exposure. CB1 and D2 protein levels in prefrontal cortex (PFC) and ventral tegmental area (VTA) were quantified using immunoblotting. CIEv reduced D2 protein levels in mSPS mice while this reduction only approached statistical significance in no stress-CIEv mice compared to respective air controls. mSPS with air exposure reduced CB1 levels compared to no stress-air controls, with this effect being reversed by CIEv exposure in mSPS mice. mSPS increased CB1 in the VTA, but mSPS and CIEv did not affect D2 receptor in the VTA. Results suggested that ethanol dependence and/or withdrawal is mediated by D2 in the PFC, and CIEv may compensate the loss of CB1 due to traumatic stress in the PFC. Additional regions that mediate traumatic stress will be examined.
The oncolytic potential of tanapoxvirus for the virotherapy of retinoblastoma

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There are currently several viruses being engineered as potential therapeutics for a wide range of different cancers. Adenovirus, poliovirus and poxviruses have shown different levels of success. We are exploring different genetically modified variants of tanapoxvirus (TPV) as a potential oncolytic therapy in multiple types of cancer, including colorectal, melanoma, and ovarian cancer. In this study, we have tested these viruses on the earliest form of childhood cancer, retinoblastoma. Here we looked at the efficacy of these engineered viruses in an in vitro model of retinoblastoma. Two different retinoblastoma tumor cell lines were used in these experiments; WERI-RB1 and Y79. These cell lines were chosen because of their wide use by other labs in the study of retinoblastoma both in vitro and in vivo. To ascertain if engineered TPV could affect retinoblastoma tumors in vitro, two different assays were used. First a cell survivability assay was used to evaluate the ability of each virus to kill the cells irrespective of the virus’s ability to replicate. A cell counting kit-8 (CCK-8) was used on virus infected cells at three different time points with two different multiplicity of infection (MOI) for each virus. Data collected demonstrated that both MOIs caused a significant decrease in living cells and demonstrated that the virus lysed the tumor cells. The second half of this study was to evaluate the ability for each variant of the TPV to proliferate. This was achieved through use of a cell susceptibility assay. This assay was used to quantitate the amount of virus that replicated during the course of viral infection. Cells were maintained over the course of three time points, 48, 96 and 240 hours, processed to lyse the cells and then the virus was titrated. As with the CCK-8 assay, two MOIs, 0.1 and 10 were used. The 0.1 assay showed an increase in virus at 240 hours, but a decrease at 96 hours. This is consistent with other tumor cells that have been assayed earlier in our lab. The 10 MOI showed a large increase between 48 hours and 96 hours, and a modest increase from 96 to 240 hours. The resulting data provides evidence that the viruses killed the cells and that the virus was able to replicate competently in retinoblastoma tumor lines. As a result, these viruses are now being used in an in vivo nude mouse model to ascertain the function of the mutated TPV under physiological conditions.
Pathological changes involving the amino-terminus of tau are associated with pathological forms of the protein and are among the first detectable alterations in Alzheimer’s disease (AD) and other tauopathies. A phosphatase-activating domain (PAD), located within this region, is aberrantly exposed in these pathological forms. This exposure initiates a signaling cascade that leads to disruption of anterograde fast axonal transport, a critical process for the maintenance and function of neurons. We have characterized four antibodies that identify the amino-terminus of tau, TNT1, TNT2 (a novel antibody), Tau12, and Tau13, in order to further study this region and its role in neurodegeneration. We refined the epitopes of these antibodies using scanning alanine mutations, determined the relative abilities of the antibodies to identify pathological tau using native conformation and denaturing assays, and examined the patterns of pathology labeled by each of the antibodies in human post-mortem AD brain tissue at various Braak stages as well as in other tauopathies. The antibodies could be classified into two basic groups. The TNT1 and TNT2 antibodies bound to tau within amino acids 7-12. They identified aggregated recombinant tau as well as soluble and insoluble forms of tau in AD brain lysates but were unable to detect monomeric recombinant tau or tau extracted from age-matched control brains in their native conformations. However, when normal and pathological forms of tau were analyzed under denaturing conditions, TNT1 and TNT2 did not distinguish between them, indicating that the differences in reactivity observed under native conditions are conformation-dependent. These antibodies also specifically labeled tau pathology in all Braak stages and in various non-AD tauopathies. These antibodies preferentially identified early pre-tangle neurons and reactivity was lost in late stage neurofibrillary tangles (NFTs). In contrast, Tau12 and Tau13 identified discontinuous epitopes in tau’s amino-terminus and, despite the close proximity to the TNT1 and TNT2 epitopes, lacked the ability to differentiate between normal and pathological forms of the protein in native or denaturing conditions. These differences suggest that conformational changes in tau, associated with PAD exposure in pathological forms of the protein, are not detected by all amino-terminal antibodies. TNT1 and TNT2 are useful for their highly-specific abilities to identify early pathological tau alterations in non-denaturing biochemical assays as well as in post-mortem human brain tissue and may help to elucidate toxic mechanisms involving aberrant PAD exposure in AD and other tauopathies.
Determination of morphine-induced gene expression in dopamine neurons using translating ribosome affinity purification (TRAP)

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Opiate drugs are the leading treatment for severe or chronic pain in the USA despite their extremely addictive properties. Chronic opiate exposure induces neuroadaptations in the mesocorticolimbic system, particularly in ventral tegmental area (VTA) dopamine (DA) neurons. VTA DA structural and functional plasticity are central to morphine reward and addiction, yet the molecular mechanisms driving these neuroadaptations remain elusive. For example, drug-induced changes in VTA gene expression have been limited to homogenization of the entire VTA, a heterogeneous region containing both DA and GABA neurons. To specifically examine gene expression changes in VTA DA neurons, we have utilized Translating Ribosome Affinity Purification (TRAP). We have crossed DA neuron Cre-driver lines, (tyrosine hydroxylase (TH)-Cre or dopamine transporter (DAT)-Cre) with Rosa26 EGFP-L10a mice, to generate THEGFP-L10a and DATEGFP-L10a mice allowing isolation of mRNA from VTA DA neurons of sham or morphine-treated mice. We first verified neuron specific EGFP expression in TH-positive neurons in the VTA of THEGFP-L10a and DATEGFP-L10a adult mice via immunohistochemistry. Next, we optimized the amount of VTA tissue and found that pooling VTA from 4 DATEGFP-L10a mice consistently yielded 1.5-2 ng RNA following TRAP. In order to assess the specificity of the purification, we analyzed expression of established DA and GABA neuron markers using RT-PCR. We found significant enrichment (2-3 fold) of TH and DAT mRNA in bound fractions compared to input control. Additionally, there was significant depletion (~80%) of GABAergic markers glutamic acid decarboxylase (GAD) and vesicular GABA transporter (VGAT) in bound fractions compared to input controls. Together, these data were consistent with DA neuron-specific mRNA in the bound fraction. We are now examining morphine-induced changes in the expression of candidate genes. THEGFP-L10a mice were subcutaneously implanted with either a sham or morphine (25 mg) pellet on days 1 and 3. The VTA was rapidly dissected on day 5, a time-point where VTA DA neuron function and morphology are altered. We will first confirm TRAP specificity via DA and GABA neuronal markers then examine candidate genes previously associated with chronic morphine exposure such as serum and glucocorticoid-regulated kinase 1 (SGK1). Additionally, future studies will use RNA sequencing to identify novel genes regulated by chronic morphine administration. We hope to uncover the molecular mechanisms that underlie opiate-induced neuroadaptations in the VTA and identify novel targets for improved therapeutics.
ENHANCED MOTIVATIONAL CONTROL OF PAVLOVIAN CUES ON FOOD SEEKING BEHAVIOR IN OBESITY PRONE RATS

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While the decision to seek out food can be controlled by endogenous hunger signals, it can also be influenced by external Pavlovian stimuli that predict food availability. Human studies suggest that enhanced sensitivity to food-cues may promote obesity and hamper weight loss in susceptible individuals. Here, we asked whether the incentive-motivational properties of a food-cue are enhanced in obesity-prone vs. obesity-resistant rats in the absence of overt obesity. In Experiment 1, we used Pavlovian-to-instrumental transfer (PIT) to assess the motivational strength of food-cues by measuring their ability to invigorate food-seeking behavior. First, rats were trained to press a lever for food. Next, they received 8 sessions of Pavlovian training where one cue (CS+) was always paired with food pellets delivery and another cue (CS-) was presented an equal number of times but was never paired with food. Following training rats were tested for PIT. In this test levers were continuously available and the cues from training were presented intermittently across the session. Lever pressing was never rewarded in this test. PIT was demonstrated by the selective invigoration of active lever pressing by the CS+, but not the CS-. During PIT testing, obesity-prone rats showed stronger conditioned approach to the food cup during CS+ presentation and expressed strong and persistent PIT relative to obesity-resistant rats. In contrast, while obesity resistant rats showed conditioned food cup approach to the CS+, they did not exhibit convincing PIT. Thus, in the absence of obesity, the ability of a CS+ to invigorate instrumental food-seeking was stronger in obesity-prone rats. These data show that individual susceptibility to obesity is linked to enhanced motivational control of Pavlovian cues, which is consistent with our recent findings in outbred rats (Robinson et al., 2015). In Experiment 2, we determined the effects of brief exposure to a sugary, fatty, “junkfood” diet on PIT. Training and testing were identical to Experiment 1, except that prior to initial training rats were given intermittent access to discrete amounts of “junk-food”. We found that exposure to “junk-food” abolished PIT, but not conditioned approach, in obesity-resistant rats. Conversely, “junk-food” exposure in obesity-prone rats resulted in a highly focused PIT effect, with responding in the presence of the CS+ almost exclusively dedicated to lever pressing, with very little approach to the food cup. Thus, in susceptible individuals junk-food exposure enhanced PIT. Together these data show that interactions between pre-disposition and consumption of fatty, sugary, foods enhance the motivational properties of food-paired Pavlovian cues and will be discussed in light of the role of altered mesolimbic reward circuits in obesity.
Afferent input is known to be critical to the maintenance of adult brain structures. The removal of sensory input to the olfactory bulb of adult zebrafish, accomplished through ablation of the olfactory epithelium, results in numerous effects including a decrease in bulbar volume. To study this effect on a cellular level, mitral cells, the primary output neurons of the olfactory bulb, were examined. The dendritic arbors of neurons are complex structures, with their shape and synaptic connections necessary for the maintenance of neuronal structure and function. Within the adult brain the cellular interactions that moderate dendritic morphology are not yet well understood. Our hypothesis is that permanent removal of sensory input will result in a decrease in complexity of the dendritic arbors of mitral cells within the olfactory bulb. We further hypothesize that temporary removal of sensory input will also decrease the complexity of dendritic arbors of mitral cells, but these effects will be reversed with reinnervation. The olfactory epithelium of adult zebrafish was permanently ablated using a small-vessel cautery iron and temporarily removed through repeated intranasal infusion with the detergent Triton X-100 every three days for 8 weeks. Mitral cells were identified using retrograde tract tracing with a fluorescent dextran applied to the olfactory tracts of whole brains in culture. Projection images were obtained using whole-mount confocal microscopy, and the dendritic arbors were traced with an image analysis program. Dendritic traces were used to quantify the total number of major dendritic branches, their length, and the size of the dendritic field. Overall dendritic complexity was determined using a modified Sholl analysis. Following 6, 8, and 20 weeks of permanent deafferentation there were significant reductions in the total number of dendritic branches, the length of those branches, and the size of the dendritic field compared to internal control (p<0.05). Following 8 weeks of temporary deafferentation there were similar significant decreases (p<0.05), and following 8 weeks of recovery the number of major branches, length of major branches, and size of the dendritic field returned to near control levels (p>0.05). Sensory innervation is critical for the maintenance of mitral cell dendritic morphology in the adult zebrafish, and this study provides a model that will allow for future investigations into dendritic plasticity and the potential for recovery of output neurons in the adult brain following injury or disease.
Nato3 overexpression induces ectopic expression of shh, foxa2, and lmx1b in the midbrain of the developing chick embryo

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In Parkinson's disease, mesencephalic dopaminergic neurons within the pars compacta region of the substantia nigra die, and the idea of cell replacement therapy to counteract this is being investigated. There is no clear answer as to how Nato3, a basic helix-loop-helix transcription factor expressed in the developing midbrain and spinal cord, affects the generation of dopaminergic neurons in the developing nervous system. Previous studies have investigated the necessity of Nato3 in vivo, but have failed to address if Nato3 was sufficient to affect neural stem cell fates. To expand upon the previous results, we explored the effects of an overexpression of Nato3 in the developing chick embryo. The examination resulted in an understanding that overexpressing Nato3 sufficiently induces ectopic expression of the floor plate cell markers Shh and Foxa2 in the developing midbrain, as well as the immature dopamine neuron marker Lmx1b in the developing midbrain and spinal cord. The results were obtained through the use of in ovo electroporation of a bicistronic EGFP reporter expression vector, and immunohistochemistry. With this new information, it is evident that Nato3 affects cells within a dopaminergic neuron lineage. Further characterization of these effects could identify possible uses related to the development of dopaminergic neurons for cell therapy.

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Drugs of abuse alter SGK1 phosphorylation and activity in the ventral tegmental area

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Drugs of abuse are known to regulate activity of the mesolimbic dopamine system. Specifically, within the ventral tegmental area (VTA) changes in cellular activity and gene regulation caused by drugs of abuse contribute to behavioral outputs that characterize drug seeking and abuse. Previous work from our lab has shown that serum- and glucocorticoid-inducible kinase 1 (SGK1) phosphorylation and catalytic activity are increased by chronic but not acute administration of cocaine and morphine. Increased phosphorylation of SGK1 and its substrate N-myc downstream regulated gene (NDRG) are observed 1 hour, but not at 24 hours, post-injection. However, the functional significance of these changes in drug-related behaviors remains unclear. Our lab has created viral constructs that will be used to mimic or prevent phosphorylation at specific SGK1 residues, and the behavioral relevance of these changes will be assessed. Specifically, we are investigating the role of phosphorylation at Ser78, which is increased by drugs of abuse, as well as the canonical S422 and T256 sites. We will evaluate voluntary drug intake using a morphine two-bottle choice task, and drug reward via conditioned place preference for both cocaine and morphine. In addition to looking at the effect of SGK1 activity modulation while cocaine and morphine are on board, we are also interested in how VTA SGK1 activity is altered following abstinence and drug re-exposure. To address this question, we injected mice with saline, cocaine, or morphine for 7 days and mice were subjected to 6 days of abstinence before receiving a challenge dose of saline or drug. VTA was microdissected from these animals and processed for western blot analysis. Surprisingly, significant increases in SGK1 phosphorylation were observed in mice with previous drug experience that received saline, but not drug, on challenge day. Future studies will assess whether this change is dependent on drug context and will utilize viral vectors to determine whether changes in VTA SGK1 signaling influence drug seeking or craving. Thus, the goal of these studies is to characterize the role of VTA SGK1 activity in drug-related behaviors to better understand the neuroadaptations that contribute to drug addiction.
Obesity is a growing epidemic with over one third of the U.S. adult population being obese. While homeostatic mechanisms play a role in obesity, it is becoming clear that overconsumption is often influenced by areas of the brain involved in reward and executive functions, such as working memory, decision-making and inhibitory control. The prefrontal cortex (PFC) is central to these functions and integrates information from multiple brain areas involved in processing interactions with food. PFC circuitry has been shown to influence self-control mechanisms in response to hedonic feeding, the drive to obtain reward beyond homeostatic need. Impaired functioning of the PFC can lead to deficits in executive control, including working memory. We hypothesize that consumption of a “junk-food” diet will disrupt PFC pathways involved in hedonic feeding. To examine the effects of a junk-food diet on the PFC, we utilized Sprague-Dawley rats to perform behavioral and protein analyses. Following a high fat diet, rats underwent working memory tests including spontaneous alternation and the Morris Water Maze. Preliminary results suggest that a junk-food diet may lead to PFC deficits.
**Ga\textsubscript{0} Proteins Mediate the Antihyperalgesic and Antidepressant-like Effects, but not the Convulsive Effects, Produced by Delta Opioid Receptor Activation**

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The delta opioid receptor (DOPr) is a member of the opioid receptor family of G protein-coupled receptors (GPCRs). Activation of DOPr induces antihyperalgesia and antidepressant-like effects in animal models. However, some DOPr agonists cause convulsions, hindering their development as therapeutics in humans. Regulator of G protein signaling (RGS) proteins act as negative modulators of GPCR signaling. Our lab has previously demonstrated that RGS4 acts as a negative modulator of DOPr-mediated antihyperalgesia and antidepressant-like effects without impacting convulsions. These findings indicate that the antihyperalgesic and antidepressant-like effects of DOPr are mediated by G proteins. However, DOPr is known to couple to multiple G\textsubscript{i/o} proteins. Therefore, we sought to determine which G protein mediates these behaviors and whether that G protein also plays a role in generating DOPr-mediated convulsions.

To this end, we compared behaviors induced by the DOPr agonist SNC80 in Ga\textsubscript{0} RGS-insensitive (RGSi) knock-in, and Ga\textsubscript{0} heterozygote knockout mice. DOPr-mediated antihyperalgesia was evaluated using a CNS-mediated nitroglycerin-induced thermal hyperalgesia assay. Antidepressant-like effects were measured using the forced swim test. The potency of SNC80-induced antihyperalgesia was significantly increased in the Ga\textsubscript{0} RGSi mice. SNC80 also produced antidepressant-like effects in the Ga\textsubscript{0} RGSi mice with enhanced potency and efficacy. In the Ga\textsubscript{0} heterozygote knockout mice, SNC80-induced antihyperalgesia was completely abolished while antidepressant-like effects in the forced swim test were unaltered. There were no changes in the frequency or severity of SNC80-induced convulsions in either mouse strain.

Taken together, our data demonstrate that DOPr-mediated antihyperalgesia and antidepressant-like effects signal through Ga\textsubscript{0}. A 50% loss in Ga\textsubscript{0} was sufficient to abolish the antihyperalgesic, but not the antidepressant-like effects of DOPr, indicating that DOPr-mediated antidepressant-like effects have a lower efficacy requirement relative to the antihyperalgesic effects. Ostensibly, DOPr-mediated convulsions are not regulated by Ga\textsubscript{0}. However, it is possible that the efficacy requirement for convulsions is low enough that they are unaffected by loss of RGS regulation or a 50% reduction in Ga\textsubscript{0}. DOPr-mediated convulsions may also be generated by a G protein-independent signaling mechanism such as the β-arrestin pathway. Future studies will focus on investigating the efficacy requirements of DOPr-mediated behaviors and characterizing these behaviors in β-arrestin2 knockout mice.
Development of Novel Methods for Altering and Measuring Mouse Olfactory Behavior

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Olfactory sensory neurons of the nasal cavity project environmental chemical information to the olfactory bulb (OB) for processing. The OB consistently alters neuronal physiology in response to changing environmental stimuli. This alteration is known as plasticity, and is commonly identified by a drop in dopamine following a loss of sensory activity. The cellular mechanisms of plasticity, and the effects on behavior, remain unknown. In the present study, we sought to measure changes in mouse olfactory behavior following artificial and temporary loss of sensory capabilities when treated with surfactant Triton-X. In addition, we sought to cost effectively retrofit a commercial behavioral olfactometer to reliably measure animal olfactory behavior such as odor detection and discrimination.

We first established a manual habituation – dishabituation behavioral assay called a Q-tip task to measure animal behavior with and without triton treatment (Intranasal irrigation, 0.1% Triton bilaterally). Naïve animals investigated 5 trials of scented cotton swabs placed within their cage. The first four trials of the q-tip task are blank odors (10ul mineral oil) followed by a scented test trial (typically 1:1000 acetophenone in mineral oil). Investigation time during each trial is recorded. Animals were considered able to perceive the test odor if investigation was greater in the 5th trial over the 4th trial. Triton animals were unable to detect the test odor (n = 4, p < 0.05), establishing our ability to manipulate mouse behavior. We then automated our task using a modified Vulintus behavioral olfactometer to produce equivalent habituation - dishabituation responses. Increased poking behavior and latency to first poke were measured. For controls, untreated mice remained habituated when presented identical odorants for the 4th trial and test trial, and dishabituate when the test trial odor presented is novel to the 4th (n = 12, p-value = 0.1, n = 14, p-value < 0.05), validating the animals responded exclusively odors presented. The manual task treatment groups were replicated for testing automated equipment sensitivity. Triton mice remained habituated to the test odor when compared to our PBS control (n = 6, p < 0.05, n = 6, p > 0.05). These mice were also histologically analyzed for altered olfactory bulb tyrosine hydroxylase levels. Triton treated mice had a 49.48% decrease in TH positive cells compared to PBS control (n = 12, p < 0.05, Cohen’s d 1.846). We then sought to measure odor detection of Amyl-acetate (AA), a typical odorant used, with a reported threshold concentration of 1E-6 AA:Mineral oil. In our task, mice respond to concentrations of 1E-8 (n = 8, p < 0.05). Mice also spontaneously dishabituate during the mineral oil habituation trials. We are unclear on why we received these results. Interestingly, using a separate vial for each odorant trial appears to normalize irregular behavior. Unlike other published tasks, latency was measured, and our results show quick response (~5s) to odorants reported undetectable. Perhaps animals unconstrained by motivational state can respond to cues that do not necessarily encode odor identity. Further investigation into latency as a response metric may help answer this.
NEUROINFLAMMATION PARALLELS A-SYNUCLEIN ACCUMULATION
AND PRECEDES NIGRAL DEGENERATION INDUCED BY
INTRASTRIATAL INJECTION OF PREFORMED α-SYNUCLEIN FIBRILS

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Neuroinflammatory markers are observed in post mortem Parkinson’s disease (PD) brains, and longitudinal PET imaging reveals early microglial activation in the basal ganglia of PD patients. α-synuclein (α-syn) activates microglia in vitro, suggesting that α-syn may participate in PD neuroinflammation. However, it remains unclear whether inflammation contributes to nigral degeneration or is merely a secondary consequence of degenerating neurons. Historically, one difficulty in delineating this sequence of events is the lack of an animal model that displays both α-syn aggregation and nigral degeneration in a protracted time course. Our lab recently characterized the accumulation of phosphorylated α-syn (pSyn) intraneuronal inclusions and bilateral nigrostriatal degeneration following intrastriatal injection of mouse α-syn preformed fibrils (PFFs) into rats. α-syn PFFs are taken up by striatal terminals and seed endogenous α-syn conversion into a pathological hyperphosphorylated form resulting in widespread Lewy-body like pathology and ~40% substantia nigra (SN) dopamine neuron degeneration over 6 months. To examine the neuroinflammatory signature in this model male Fischer344 rats received unilateral intrastriatal injections of mouse α-syn PFFs or saline. Cohorts of rats (total n=96) were euthanized at various times post-injection (0.5, 0.75, 1, 2, 3, 4, 5 & 6 months). Outcome measures at each time point included quantification of tyrosine hydroxylase positive (TH+) neurons, phosphorylated α-syn (pSYN+) aggregates, microglial density and major histocompatibility complex-II antigen-presenting microglia (MHC-II+) in the SN. Both intraneuronal pSYN+ inclusions and MHC-II+ microglia peaked between 1-2 months following PFF injection, and decreased over time in association with SN dopamine neuron loss. By month 3 significantly fewer pSYN+ inclusions were observed in the SN ipsilateral to injection, compared to 2 months (p < 0.05). By month 4 significantly fewer MHC-II+ microglia were present in the ipsilateral SN (p <0.05). Few to no MHC-II+ microglia were observed in the contralateral SN at any time point or in the ipsilateral SN of saline-injected control rats at 2 months and beyond. Additional analysis of MHC-II+ microglia in the striatum (ST) and microglial density and inflammatory cytokines in both the ST and SN are ongoing. These initial results demonstrate that neuroinflammation precedes the loss of SN dopamine neurons induced by α-syn aggregation and suggests that α-syn-mediated neuroinflammation may contribute to the degenerative process.

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ACTIVITY-DEPENDENT TRANSCRIPTIONAL REGULATION OF HIPPOCAMPAL PROJECTIONS IN FEAR/ANXIETY AND COCAINE REWARD

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Abstract:
Drug seeking, a hallmark of addiction, is mediated by neurobiological adaptations in the reward circuitry of the brain. In particular, dysfunctional regulation of the nucleus accumbens (NAc) by the hippocampus (HPC) promotes the development of drug addiction, possibly via enhanced drug reward, and evidence suggests this is mediated by direct glutamatergic projections from ventral HPC (vHPC) to NAc. In addition, the vHPC is important for regulating fear and anxiety behavior via projections to the amygdala (Amy). These projection neurons may also undergo significant functional adaptations after learning, stress, or exposure to drugs of abuse, such as cocaine, resulting from activity-dependent alterations in transcription. \(\Delta FosB\), encoded by the \(FOSB\) gene, is a chronic activity-dependent transcription factor due to its exceptionally long half-life \textit{in vivo}. However, the mechanisms underlying HPC projection-specific changes in gene expression driven by activity-dependent transcription factors, such as \(\Delta FosB\), are not understood. We aimed to specifically determine the role of \(\Delta FosB\) in HPC projections to NAc and Amy and their associated outputs of behavior: cocaine reward and fear/anxiety, respectively. We developed a novel dual-viral CRISPR-Cas9 technique to interrogate projection specific gene expression that allowed us to selectively mutate the \(FOSB\) gene, which encodes for the expression of \(\Delta FosB\), thereby creating a functional \(FOSB\) knockout (KO) in HPC projections to NAc or Amy in adult mice. We first demonstrate here that non-specific \(\Delta FosB\) inhibition in the ventral HPC (vHPC) impairs cocaine conditioned place preference (CPP), which measures the expression of drug reward. Next we examined the effects of \(FOSB\) KO in specific vHPC projections on cocaine CPP and avoidant learning, which are mediated by the NAc and Amy, respectively. We found that \(FOSB\) in HPC-NAc projections was important for the expression of cocaine CPP, but not avoidant learning. Conversely, \(FOSB\) KO in HPC-Amy projections impaired avoidant learning and reduced anxiety-like behavior, but did not affect expression of cocaine CPP. These findings suggest that \(\Delta FosB\) regulates projection-specific HPC neuron functional adaptations via changes in gene expression, thereby driving cocaine-dependent behaviors and stress-evoked behaviors, such as fear & anxiety. Furthermore, they suggest that \(\Delta FosB\) in HPC projections may be important in drug addiction and stress-related psychiatric disorders, such as posttraumatic stress disorder and depression.

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5-HT₃ receptor signaling in a rat model of sex specific visceral hypersensitivity
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Introduction/ Background. The Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder characterized by altered bowel habits and visceral hypersensitivity, especially in women. 5-Hydroxytryptamine (5-HT, serotonin) signaling is disrupted in some IBS patients possibly due to polymorphic variations in the gene encoding the serotonin transporter (SERT) which result in increased extracellular 5-HT availability. Female SERT knockout (KO) rats exhibit visceral hypersensitivity to colonic distention that mimics colonic hypersensitivity known to occur in female IBS patients. Alosetron, a 5-HT₃ receptor antagonist is FDA approved for the treatment of IBS in women suggesting that 5-HT signaling at 5-HT₃ receptors plays a role in the pathogenesis of IBS. Aims. We tested the hypothesis that 5-HT action at 5-HT₃ receptors contributes to visceral hypersensitivity. Methods. We examined the effects of acute subcutaneous (s.c.) and intrathecal (i.t.) administration of morphine and the 5-HT₃ receptor antagonists alosetron, granisetron, and ondansetron on visceral sensitivity in SERT KO and wild type (WT) rats. We measured the visceromotor response (VMR) to colorectal distension (CRD) Results. Ondansetron (0.1 mg/kg, s.c.) did not affect visceral sensitivity, while alosetron (0.1 mg/kg, s.c.) increased the VMR to CRD in SERT KO female rats and WT male rats. Granisetron (0.1 mg/kg, s.c.) increased VMR to CRD in SERT KO female rats only. Morphine (3 mg/kg s.c.) suppressed the VMR to CRD in all rats. Ondansetron (25 nmol, i.t.) and Granisetron (25 nmol, i.t.) did not affect visceral sensitivity, while alosetron (25 nmol, i.t) increased the VMR to CRD in SERT KO female. Morphine (10 µg, i.t.) suppressed the VMR to CRD in all rats. Conclusion. The 5-HT₃ receptor antagonists used cross the blood brain barrier. Therefore, the results suggest that global 5-HT₃ receptor antagonism causes a paradoxical increase in the VMR to CRD in SERT KO female rats and WT male rats. The increase in VMR to CRD observed with inhibition of 5-HT₃ receptors at the level of the spinal cord in SERT KO female rats suggest that in the presence of altered serotonergic singling, ovarian hormones might modulate this response.

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Autophagy in Parkinson Disease: An Evaluation of Markers of Autophagy in WT and Parkin Deficient Mice
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The balance between protein production and degradation is an important part of maintaining cell homeostasis and survival. The ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP) are two pathways responsible for the turnover of certain substrates and removal of abnormal proteins. Efficient protein catabolism is necessary for cell survival because an accumulation of non-functional proteins can lead to aggregation and toxicity often seen in neurodegeneration. Failure of the UPS or ALP to degrade misfolded and aggregated proteins has been identified in the pathogenesis of many disease states such as Parkinson disease (PD), the second most common neurodegenerative disorder. Impairment of the UPS has been one primary focus in studying the importance of protein quality control in PD, yet there is an increasing interest in the function of the ALP in neurodegeneration. Parallels between the two systems have been revealed because they both degrade disease-associated proteins such as α-synuclein. These links suggest the UPS and autophagy may work closely together to maintain proper protein degradation. Accumulation of ubiquitinated protein aggregates found in diseased neurons is a characteristic of PD. Interestingly, structures related to autophagy are also found in affected neurons. Autophagosomes are often observed in dying neurons of PD patients and in animal models of PD. Proteasome activity is hindered in parkin knock out mice (Lansdell, 2015), and preliminary studies in our lab show that LC3B-II is elevated in the absence of parkin. This evidence suggests that when the UPS is decreased in the absence of parkin, an increase in autophagy could occur. The upregulation of autophagy in dying neurons could be a compensatory mechanism to keep up with proper protein degradation after the UPS is damaged. The predicted compensatory relationship between autophagy, the ubiquitin proteasome system, and relationship between loss of parkin and autophagy regulation will be explored in this study by comparing wild type to parkin deficient mice. Indices of autophagosome maturation, chaperone-mediated autophagy, specific substrates of autophagy, and K63-linked polyubiquitin chains will be measured to determine if parkin KO mice show compensatory upregulation of autophagy in the face of UPS impairment.
Dopamine neurons are important elements of the mesocorticolimbic reward circuit and are thought to play a role in reward prediction error. It has also been argued that the dopaminergic system is involved in incentive salience attribution, that is, the attribution of motivational value to reward predictive cues. We compared these two ideas by recording from dopamine neurons from rats performing a Pavlovian approach task (Flagel et al., 2007), which contain cues that have both “predictive” and “incentive” properties. In this behavioral model, a conditioned stimulus (CS) consisting of an illuminated lever is presented for 8 seconds (predictive cue) at random intervals followed by retraction of lever (incentive cue) and delivery of a food reward (unconditioned stimulus, US). All animals learned the predictive nature of the cue (illuminated lever entry into cage), but some also found the cue to be attractive and motivated their attention towards it. Rodents that preferentially approach and interact with the lever itself (“sign-trackers”, STs) were compared to animals that predominantly approach the location of reward receptacle (“goal-trackers”, GTs). Following training to determine phenotype, rats were implanted with tetrodes for neural electrophysiological recordings in the ventral tegmental area (VTA). Dopamine cells were characterized by spike waveform shape and firing rate (Roesch et al., 2007) and in 8 sessions, the dopaminergic nature was confirmed by a systemic apomorphine (0.75 mg/kg) test. Electrode bundle placement was confirmed histologically. Firing rates and magnitudes of responses in relation to Pavlovian behaviors, cue presentation, and reward delivery were assessed. STs are thought to place incentive salience on reward-paired cues while GTs do not. We hypothesize there should be a deeper modulation of neural activity (i.e. coding) in STs than GTs to incentive cues.

We identified 89 dopamine and 90 non-dopamine neurons. GTs and STs both showed responses to the initial (400ms) lever presentation (CS1) and also lever retraction (CS2). However, higher firing rates were sustained during the 8s lever presentation, the interaction period, only in STs, while they interacted with the lever. Even though GTs interacted just as vigorously with the goal there was no concomitant neural activation indicating that motor differences do not account for the findings. Further, dopamine cells of STs showed a significantly higher proportion of cells responding to cue offset, the incentive cue, than to cue onset, the predictive cue. Neurons outside the VTA were not responsive to the task. These are the first results to show that neurons from the VTA encode both predictive and incentive cues. These results support an important role for dopamine neurons in the attribution of incentive salience to reward-paired cues and underscore the consequences of potential differences in motivational behavior between individuals.
PREVALENT BDNF RS6265 VARIANT DELAYS PROGRESSION AND PREDICTS RESPONSE TO DOPAMINERGIC THERAPY IN EARLY-STAGE PARKINSON’S DISEASE

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While most Parkinson’s disease (PD) patients lack a potent genetic cause like Park8 or Snca mutations, 'precision medicine' promises that genetic factors will predict a patient’s course and responses to different therapies. A prevalent variant in the gene Bdnf (rs6265) reduces activity-dependent release of brain-derived neurotrophic factor (BDNF), a protein with critical roles in plasticity-dependent processes and efficacy of several pharmacotherapies. We examined whether the rs6265 variant alters: 1) progression in early-stage PD or 2) efficacy of pharmacological versus surgical therapy in early-stage PD.

To examine PD progression, a retrospective analysis was conducted on early-stage, unmedicated PD subjects from the DATATOP trial (n = 217). Subjects were genotyped for the Bdnf variant rs6265 and the time to initiate dopaminergic therapy was assessed. PD subjects carrying both minor alleles (Met/Met) of the rs6265 variant conferred a significant delay in the initiation of dopaminergic pharmacotherapy (p = 0.02, HR = 0.2).

To examine response to pharmacological and surgical therapies, early-stage PD subjects (n = 28) from the “Vanderbilt DBS in Early Stage PD” trial (NCT00282152) were genotyped. Retrospective analyses were conducted to compare the responses to dopaminergic drug therapy versus subthalamic nucleus deep brain stimulation (STN DBS). No differences were observed at baseline; however, minor allele carriers randomized to drug therapy displayed significantly worse UPDRS, UPDRS-II and PDQ-39 scores starting at 12 months and continuing through two years (p < 0.05). The rs6265 variant did not affect subject response to STN DBS.

Genotyping for the Bdnf variant rs6265 may predict disease progression and inform optimal treatment strategies in early-stage PD. Validation of these findings in larger cohorts of PD subjects would support genotyping for rs6265 variant status for use in patient-specific counseling at diagnosis, investigation of personalized treatments and efficient design of clinical trials.

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Intrastriatal Transplantation of Mouse Adenovirus-Generated Induced Pluripotent Stem Cells Reduced Behavioral Deficits in the YAC128 Mouse Model of Huntington’s Disease

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Huntington’s disease (HD) is an inherited neurodegenerative disorder with no known effective treatment to delay its onset or progression. However, the use of induced pluripotent stem cells (iPSCs) holds significant promise as a potential treatment. The ultimate goal of our research is to replace the HD-induced loss of striatal neurons with iPSC-derived neurons and to preserve or restore normal functional outcomes. To this end, we developed mouse adenovirus-generated iPSCs and transplanted them into striata of wild-type (WT) and HD YAC128 mice. We then tested the efficacy of these transplanted iPSCs for (1) their ability to survive and differentiate into neuronal phenotypes (without forming tumors) and (2) their ability to reduce the significant motor deficits observed in this mouse model of HD.

To assess the survivability, differentiation potential, and safety (potential tumor formation), we bilaterally transplanted iPSCs, which were pre-labelled with Hoechst 33342, into the striata of female WT and HD YAC128 mice at 12-months age. Four- or eight-weeks following transplantation, the mice were perfused, and their brains were frozen, sectioned and analyzed by immunohistochemistry. Our results revealed that iPSCs in both WT and HD mice had survived and showed evidence of differentiation into neuronal phenotypes, with co-labelling of Hoechst with Tuj1 and NeuN at both the 4- and 8-week post-transplant time points. In addition, we observed a reduction in reactive astrocytes (GFAP+) at 8-weeks, compared to the 4-weeks, at the site of transplantation and infiltrating areas where surviving cells were located. We also did not see formation of tumors in the brains.

To further analyze the functional efficacy of iPSC transplantation, male and female WT and HD YAC128 mice were bilaterally transplanted at 10-months of age with iPSCs, pre-labelled with Hoechst 33342, or vehicle control. Accelerod, open field, and clasping behavioral testing were performed one day before the transplantation, and then weekly for 10 weeks. The repeated-measures ANOVAs and Tukey post hoc tests revealed significant differences for accelerod data between WT and HD mice for the vehicle control groups. Interestingly, there was an amelioration of locomotor deficits for the HD group that received iPSC transplantation. Open field and clasping data for all groups at baseline and week 10 showed no significant difference for total movement.

Collectively, our histological and behavioral data suggest that adenovirus-generated iPSCs may provide a safe and effective option for neuronal replacement therapy. Future research looking at the electrophysiological profiles of these iPSCs will be performed, which may further elucidate the mechanisms underlying the observed behavioral sparing produced by these iPSC transplants.

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Epigenetic regulation of the *FosB* gene in hippocampus

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Elucidation of the molecular mechanisms of memory formation is critical for the development of a cohesive theory of memory. However, the patterns of gene regulation that occur during learning remain unknown. Consolidation of explicit memories occurs through synaptic plasticity in the hippocampus, and some of the molecular mechanisms of this process are well characterized, but epigenetic regulation underlying changes in hippocampal gene expression and the distribution of gene expression through the hippocampus is poorly understood. The transcription factor DeltaFosB is an important arbitrator of activity-dependent gene expression in nucleus accumbens (NAc) underlying maladaptive changes in reward processing. Previous studies demonstrate that drugs of abuse and stress also upregulate DeltaFosB in hippocampus, but its role in this brain region is less understood. Recently, we published our findings that DeltaFosB is upregulated in the dorsal hippocampus in response to novel environment exposure, spatial learning, and cocaine exposure, and we are currently determining whether the expression pattern of DeltaFosB differs throughout hippocampal subregions. Furthermore, we use chromatin immunoprecipitation to demonstrate that dimethylation of lysine 9 at histone H3, a repressive histone modification, is decreased at the *FosB* gene promoter in hippocampus after exposure to either cocaine or a novel environment. We have gathered further preliminary data demonstrating that locus specific modification of the histone mark in hippocampus is sufficient to impair learning and memory. These findings collectively suggest that specific salient stimuli, such as spatial learning or drug exposure induce epigenetic changes in the hippocampal *FosB* gene promoter that regulate DeltaFosB induction, which in turn may control the transcription of genes that underlie hippocampal cell function, plasticity, and learning.
Sex Differences in Sympathetic Neurotransmission in Normotensive Rat Mesenteric Arteries

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Hypertension is less common in premenopausal women than in men possibly due to high estrogen levels in females. The diameter of mesenteric arteries (MA) is an important determinant of system blood pressure and sympathetic nerve activity is a major determinant of MA diameter. Sympathetic nerve supplying MA uses norepinephrine (NE) and ATP as vasoconstrictor transmitters in male rats. We tested the hypothesis that noradrenergic/purinergic neuroeffector transmission to MA also occurs in female rats. Young (10-14 week), normotensive Sprague Dawley male and female rats were used. The estrus cycle stage in female was determined cytologically by vaginal lavage before euthanasia. MA (200 – 250 μm inner diameter) were harvested in a physiological buffer solution and mounted in a pressure (60 mmHg) myograph and inner diameter was measured continuously. Electrical field stimulation (30 stimuli at 0.2-30Hz) and drug addition to the chamber were used to constrict MA. Neurogenic constriction was greater in male compared to female arteries. Neurogenic constriction did not vary with the estrus cycle in female rats. Neurogenic constrictions were blocked by combined application of prazosin (0.1 μM, α1-adrenergic receptor antagonist) and PPADS (10 μM, P2X-purinergic receptor antagonist) as well as suramin (100μM, P2X and P2Y-purinergic receptor antagonist) in arteries from male and female rats. PPADS/Suramin inhibited ATP and α,β-methylene ATP, but not KCl, -induced MA contraction. Constrictions caused by exogenous NE, ATP α,β-methylene ATP or elevated KCl were similar in MA from male and female rats. Immunostaining for tyrosine hydroxylase (a sympathetic nerve marker) revealed no observable differences in nerve density and distribution in MA from male and female rats. Our data indicate that there are fundamental differences in the mechanisms of neurogenic vasoconstriction in MA of male and female rats. Differences in neurotransmission and/or nerve fiber distribution in male and in female rats might contribute to and explain the sex differences in hypertension prevalence between human males and females.
Defining Neuromuscular Measures of Development and Behavior in the Fly (Drosophila virilis) Following Thiouracil Exposure

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Endocrine disrupting compounds are those that exert effects on aspects of the neuroendocrine system in organisms, and may alter an organism's physiology, morphology, development and behavior. Thiouracil is a compound used in human medicine since the early 1940's to treat Grave's Disease. Grave's Disease is an autoimmune disease that affects the thyroid. It frequently results in hyperthyroidism and an enlarged thyroid gland. As a thyrotoxic agent, thiouracil inhibits oxidation of iodine leading to suppressed or inhibited thyroxine production in the body. This study assesses the effects of thiouracil in the fruit fly (Drosophila virilis). As reported in the literature, there is a correlated action between mammalian thyroid hormones and the sesquiterpenoid juvenile hormone (JH) seen in insects. This related action appears due to similarities in the chemical structure of these hormones and their likely ability to stimulate similar receptors at the cellular level. Physiologically relevant exposures to thiouracil could induce effects on the timing of growth, development and behavior in the invertebrate system. Specifically in this presentation, focus is on how thiouracil exposure has affected the developmental timeline of the fruit fly associated with a variety of neuromuscular assessments at different metamorphic stages with comparisons of rover/sitter behavior in larvae, pupation heights in pupae, and locomotor measures in adult flies.
Using mediated learning to examine hedonic hallucinations in a mouse model of neuropsychiatric illness

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Hallucinations are a core symptom of schizophrenia and are also encountered in mood disorders, Alzheimer’s disease and Parkinson’s disease. Sufferers of hallucinations experience a distortion between reality and what stimuli are being cognitively perceived. Pavlovian learning offers a possible mechanism of investigation via mediating learning, through which a Pavlovian cue can evoke a detailed sensory representation of an appetitive outcome (e.g., sucrose solution), despite the outcome not being presented. We have developed a paradigm to examine ‘hedonic hallucinations’ in a mouse model of neuropsychiatric illness—disrupted in schizophrenia 1 (DISC1). These mice express putative dominant negative DISC1 under expression control of the prion protein promoter (DN-DISC1), resulting in expression throughout the central nervous system. In the current study, DN-DISC1 mice were trained to respond to an extensively and minimally trained cue, both paired with a reward of sucrose solution. At the test stage, we replaced the sucrose solution with water and exposed the mice to either the extensively or minimally trained cue. Unlike controls, DN-DISC1 mice responded to water in a comparable manner to how they previously responded to sucrose. These results suggest that it is possible to examine impaired reality testing in mice, and using a genetic mouse model of neuropsychiatric illness, these ‘hedonic hallucinations’ appear to be particularly prevalent. Thus, this study provides the foundation for examining the neurobiological pathology underlying hallucinations.
Changes in environmental conditions often result in changes in the display of circadian rhythmicity and locomotor activity levels of mammals. In previous experiments, day active (diurnal) grass rats (*Arvicanthis niloticus*) have been shown to switch to a night active (nocturnal) pattern of activity after the introduction of a running wheel. However, it is not yet known the mechanism by which animals switch from being diurnal to nocturnal. Here, we used grass rats to examine activity levels following manipulations of varying ambient temperatures and lighting intensities. Animals were singly housed with running wheels and data were collected in 12:12 light-dark (LD) conditions. First, we examined how a warmer ambient temperature during the day (25 degrees Celsius) and a cooler night (21 degrees Celsius) would affect wheel running activity. We found that 100% of grass rats in this condition were diurnal. The ambient temperature was then raised to a warmer condition (constant 32 degrees Celsius). Diurnality was still expressed by 100% of the subjects following the temperature increase, yet overall wheel running activity significantly decreased ($p < .05$). Next, we reduced the ambient temperature to a colder condition (constant 15 degrees Celsius). Again, grass rats maintained diurnal patterns of activity in cold conditions. Finally, we adjusted the room temperature back to a baseline temperature of 25 degrees Celsius, and dimmed the intensity of light in the environment. Altogether, we found that changes in ambient temperatures affected overall activity levels while maintaining diurnality, whereas dimming the lights significantly affected the display of diurnal activity patterns in a subset of animals. Our results will allow us to predict how lighting and temperature maintain diurnal patterns, which is important in light of the growing evidence that humans that become night-active have significant health consequences.

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Trauma
tic stress exposure during adolescence increases resilience to fear-based behaviors during adulthood.

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Post-traumatic stress disorder (PTSD) is a debilitating mental condition that is defined by avoidant behavior, anxiety, and hyper-reactivity to trauma-associated stimuli. These behaviors are seen in rats that have been exposed to traumatic stress. However, stress sensitivity varies over the lifespan, and the behaviors of adolescents exposed to stress have been shown to be different from those of adults. To determine how the consequences of traumatic stress vary during development, we exposed adolescent rats to a series of traumatic stressors in the Single Prolonged Stress (SPS) model, and determined how early-life traumatic stress affects PTSD-like behaviors over the course of development into adulthood.

Twenty male Sprague-Dawley rats were used in this study. Ten adolescent-age rats (postnatal day 31, P31) were exposed to SPS, which included a 2 hour restraint, 20 minutes of group forced swim, and exposure to ether until loss of consciousness, and the other ten rats (P31) were given a control treatment which consisted of brief handling. At P41, all animals were tested for short term memory via novel object recognition and anxiety-like behavior using an open field. On P42, anxiety-like behavior was tested in all animals using a light-dark box. The animals were allowed to age into adulthood and at P62 their anxiety-like behavior was again tested via an elevated plus maze. Finally, from P63-65 all rats underwent cued fear conditioning and extinction.

We found that SPS exposure during adolescence does not affect anxiety-like behavior or short-term memory performance during adolescence or anxiety-like behavior during adulthood. Interestingly, we found that rats exposed to SPS show less reactivity during acquisition of fear conditioning, normal expression of fear memories during retrieval, and enhanced fear extinction. These preliminary results suggest that undergoing a traumatic stressor during adolescence may increase resilience to fear-based behaviors during adulthood.
Characterization of the Cholinergic Synapse Between Starburst Amacrine Cells and Retinal Ganglion Cells in a Rat Glaucoma Model

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Glaucoma is a neurodegenerative disease of the retina characterized by the loss of retinal ganglion cells (RGCs), and is the second leading cause of blindness worldwide. Starburst amacrine cells (SACs) in the mammalian retina are known to release acetylcholine (ACh) onto the alpha 7 nicotinic acetylcholine receptors (α7 nAChRs) on the RGCs. Previous studies from this lab have shown that application of ACh or ACh agonists in cell culture and in an in vivo model of glaucoma provides neuroprotection against the loss of RGCs. The hypothesis of this study is that SACs provide neuroprotection to RGCs under physiological conditions but this neuroprotection is compromised in glaucoma. Published findings on the loss of SACs have used various glaucoma models and have reported inconclusive results. This study analyzes the changes in cholinergic transmission between SACs and RGCs in a rat glaucoma model at various time points after glaucoma insult. Hypertonic saline injections to the episcleral vein were performed to induce glaucoma-like conditions, causing significant increase of intraocular pressure (IOP) and RGC loss within one month in adult Long Evans rats. Immunohistochemical analysis was performed to analyze changes in SAC numbers at various time points post-surgery in a flat-mounted retinal preparation. Liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) analysis was performed to quantify changes in ACh content at the same post-surgical time points. Enzyme-linked immunosorbent assay (ELISA) was performed in order to measure changes in expression of the α7 nAChRs on the RGCs for the same time points. It was found that SAC numbers showed a significant loss at two weeks post-procedure. LC/MS/MS results showed that ACh content decreased significantly within one week after surgery. Interestingly, ELISA results also showed that α7 nAChR expression significantly decreased within one week. Results from this study indicate that α7 nAChR receptor expression, SAC numbers, and their release of ACh precede the significant loss of RGC bodies at one month. This suggests a possible neuroprotective role of ACh released by SACs onto RGCs. Further research into a potential cholinergic neuroprotective mechanism in the retina could lead to the development of a treatment for glaucoma in conjunction with current therapeutic interventions.
Ovarian hormones contribute to the onset of maternal behavior following parturition in female rodents. However at the end of pregnancy, continued exposure to the ovarian hormone progesterone inhibits maternal behavior. As progesterone inhibits maternal behavior at the end of pregnancy, we hypothesized that it may also contribute to the attenuation of maternal behavior during the postpartum period. Additionally, pup contact decreases over the postpartum period and pup contact is negatively associated with maternal anxiety. To test whether ovarian hormones are necessary for the natural attenuation of maternal behavior and maternal anxiety, we ovariectomized recently-parturient dams. Following a 4 day recovery period, we conducted home-cage maternal behavior observations four times daily from postpartum day (PPD) 7 to PPD 19. On PPD 20, we examined dams’ anxiety state using the elevated plus maze. We found that ovariectomized dams licked their pups more often than sham dams, and that ovariectomized dams spent more time in active postures than control dams. We also found no difference between sham and ovariectomized dams in their anxiety-related behavior. These results indicate that ovarian hormones during the postpartum period might function to limit the incidence of proactive, or pup-seeking, maternal behaviors. Additionally, we examined oxytocin and oxytocin receptor mRNA expression in the medial preoptic area, which plays an important role in postpartum caregiving. We found a significant decrease in oxytocin receptor mRNA expression, suggesting that oxytocin receptor expression might actually function to limit the expression of certain maternal behaviors during mid-to-late lactation.
Peptidomimetics exhibiting mu opioid receptor (MOPr) agonist/delta opioid receptor (DOPr) antagonist activity as novel pain therapeutics

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Drug discovery and development of opioid ligands has largely favored highly selective agonists/antagonists for a single opioid receptor (OPr); opioid analgesics, such as morphine, are primarily selective for and activate the mu opioid receptor (MOPr). However, evidence suggests that modulation of other opioid receptors may be beneficial in MOPr-mediated analgesia. In particular, there is evidence that simultaneous activation of MOPr with inhibition of the delta opioid receptor (DOPr) can reduce the development of morphine tolerance and dependence. Thus one strategy is to design single compounds that target both mu and delta opioid receptors (activate MOPr and inhibit DOPr) to use as potential therapeutics for the improved clinical management of pain. The compounds we have synthesized are peptidomimetics, which maintain key elements of opioid peptides vital for activity but that have small molecule-like features to provide for bioavailability, blood brain barrier permeability and longer duration of action. Our compounds have been characterized in vitro by radioligand binding to provide affinity (Kᵢ) values and for potency and relative efficacy (EC₅₀, % stimulation compared to standard agonist) using the degree of incorporation of GTPγ³⁵S into G proteins in membranes from cells expressing MOPr or DOPr. Certain compounds have been evaluated for antinociceptive activity in vivo by the mouse warm water tail withdrawal assay, as well as for development of tolerance and dependence after 5-day escalating drug treatment. Lead compounds display maximal antinociceptive activity with a long duration of action and also with a marked reduction in tolerance and dependence. Our preclinical research provides a strong foundation for the development of novel opioid analgesics with fewer adverse effects such as reduced abuse liability. Funded by DA-03910 and the Pharmacological Sciences Training Program (NIGMS-GM007767)
SPECTRAL-SPATIAL MAPPING OF VISUAL RESPONSES ACROSS A SENSORY SURFACE

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How do visual systems extract and convey behaviorally relevant information with simple eyes? Our overarching hypothesis is that two dimensional pixel arrays of simple eyes can be used to extract low resolution image features. We examined this in the experimentally accessible medicinal leech, *Hirudo*, to learn how distributed visual input is used to extract information and ultimately drive adaptive behavior. Endowed with a visual system composed of simple eyes, *Hirudo* collects visual information using 5 pairs of pigmented cephalic eyecups at the margin of the anterior sucker and a distributed, segmentally iterated array of 7 pairs of dermal sensilla positioned dorsal to ventral along the central annulus of each mid-body segment. *Hirudo* can discriminate green and near ultraviolet (UV) light (Jellies. 2014. J. Exp. Biol. 217:974). Behaviorally, *Hirudo* rotates away from ventrally presented UV light, but not dorsally presented UV light and uses the distributed array of dermal sensilla as a type of “spectral statocyst” to maintain 3-D body position (Jellies. 2014. J. Comp. Phsyiol. A 200:923). We are extending our studies to examine the electrophysiological basis of how the dermal sensilla encode visual cues. We predicted that the primary sensillar photoreceptors may be responsible for distinguishing wavelength, and that this preferential encoding of light would be mapped in a regular way across the dorsal-ventral axis. The dermal sensilla project light information to the CNS by subsidiary identifiable branches of the segmental nerves. Adult leeches were dissected to expose these nerves from ventral, dorsal, and lateral sensilla. We used extracellular recording in combination with light stimulation (using previously developed LEDs, red, green, blue, UV) to characterize sensillar responses across wavelength and luminosity. Complex responses from the population of photoreceptors in each sensillum were recorded, rectified, smoothed, and integrated for comparison. Ventral sensilla appeared to be preferentially narrowly tuned to respond best to UV light, while dorsal sensilla were broadly tuned with a green light preference. Therefore, we suggest that spectral contrast informed behavioral response is established at the sensory level, and that spectral and luminal cues are differentially mapped across the dorsal-ventral array of dermal sensilla. Thus, synaptic interactions between sensillar axons and interneurons in the CNS may underlie previously described UV evoked behaviors. It remains to be seen how this spectral-spatial mapping drives central circuitry of the leech nervous system to produce adaptive behavior.
Quoting Memorability and Differential Neural Connectivity

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Previous research efforts have illuminated many facets of the neural networks involved in reading. However, these studies rely on single-word or clause-by-clause text presentation, leading to a stilted, nonlinear reading experience. The Digital Humanities and Literary Cognition lab at MSU has sought to study reading by presenting full paragraphs of text, allowing participants to read at a natural pace that better mirrors functional reading. This pioneering fMRI experiment examined two modes of attention in reading: analytical close reading, and casual pleasure reading. PhD candidates in English (n = 18) read a chapter from Jane Austen’s Mansfield Park in an MRI scanner equipped with fMRI-compatible eye tracking. Text was presented in 32 blocks, arranged in alternating “close reading” and “pleasure reading” sections. For the close reading sections, subjects were instructed to write a short literary essay, which they completed after the scan. A unique data set of literary essays revealed quoting patterns in which subjects referred directly and indirectly to the text. BOLD responses revealed enhanced activity during the close reading condition in areas including the medial temporal gyrus, (language, memory, and word processing) and the superior temporal lobe (theory of mind). These results have allowed us to examine the nuances of reading, an essential component of humanist studies. Here, we analyze neural connectivity during reading as related to what subjects quote. We predict a stronger correlation between left medial temporal gyrus and left superior temporal sulcus in subjects who are high quoters compared to subjects who are low quoters.
Previous research suggests that probiotics such as \textit{Bifidobacterium Infantis (B-infantis)} may have beneficial health effects, including improving depressive symptoms. However, less is known about the mechanisms of the probiotic-induced changes or about potential sex differences in the response to probiotics. Therefore, the current study investigates whether chronic administration of \textit{B-infantis} to male and female rats can alleviate depressive symptoms in a corticosterone-induced rodent model of depression.

For this, 24 male and 24 female Sprague-Dawley rats received daily s.c. corticosterone (40mg/kg; to induce depressive-like behavior) or oil injections (controls) coupled with voluntary consumption of either pure Nutella (placebo) or Nutella mixed with one capsule of \textit{B-infantis} for 21 days. Animals performed the Open Field Test and Forced Swim Test I and II on days 18, 20 and 21, respectively and several blood samples were collected to investigate basal as well as stress-induced corticosterone levels.

Preliminary results show no significant difference in body weight, Open Field Test or Forced Swim Test behavior between the probiotics and placebo groups, but the expected decrease in body weight and increase in immobility in the corticosterone-treated animals. These results suggest, that \textit{B.infantis} may not be sufficient to improve depressive-symptoms in our animal model, however, a trend for weight and behavior changes in females indicates that more research is needed to better understand potential sex difference in the health effects of probiotics.
Most scientists would agree that psychiatric illness is unlikely to arise from pathological changes that occur uniformly across the entire brain. Despite this fact, the majority of transcriptomic analyses of the human brain are conducted using block-dissected tissue due to the difficulty of conducting single-cell level analyses on donated post-mortem brains. To address this challenge, we compiled a database of >3300 transcripts that were specifically-enriched in one of 10 primary brain cell types within published single-cell transcriptomic experiments. Using this database, we predicted the relative cell type composition for 157 human dorsolateral prefrontal cortex samples using Affymetrix microarray data from the Pritzker Neuropsychiatric Consortium, as well as for 841 samples spanning 160 brain regions included in an Agilent microarray dataset from the Allen Brain Atlas. These predictions were generated by averaging normalized expression levels across the transcripts specific to each primary cell type to create a “cell type index”. Using this method, we determined that the expression of cell type specific transcripts identified by different experiments, methodologies, and species clustered into three main cell type groups: neurons, oligodendrocytes, and astrocytes/support cells. Overall, the principal components of variation in the data were largely explained by the neuron to glia ratio of the samples. The relative balance of these cell types was influenced by a variety of demographic, pre- and post-mortem variables. In particular, age and prolonged anaerobic conditions around the time of death were associated with decreased neuronal content and increased astrocytic and endothelial content in the tissue, replicating the known vulnerability of neurons to adverse conditions and illustrating the proliferation of vasculature in a hypoxic environment. We also found that the red blood cell content was reduced in individuals who died in a manner that involved exsanguination. When comparing across brain regions, we were able to easily capture canonical cell type signatures – increased endothelial cells and vasculature in the choroid plexus, oligodendrocytes in the corpus callosum, astrocytes in the central glial substance, neurons and immature cells in the dentate gyrus, and interneurons in the body of the caudate. Finally, by including a set of “cell type indices” in a larger model examining the relationship between gene expression and neuropsychiatric illness, we were able to identify more provocative candidate molecules in relationship to major depressive disorder, bipolar disorder, and schizophrenia than using standard methodology.
Understanding the relationship between gender differences in behavior and pathology relating to heat shock protein and amyloid beta protein expression in the 5xFAD mouse model of Alzheimer’s Disease

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Alzheimer’s disease (AD) is an intricate protein misfolding neurodegenerative disorder, largely characterized by the protein misfolding and seeding of amyloid beta (Aβ) and tau proteins. There are a myriad of risk factors associated with developing AD, with age being the number one factor. Although this is true, gender is also a risk factor of concern with the proportion of women-to-men diagnosed with AD skewed in the direction of including more women. Heat shock protein 70 (Hsp70) has the ability to bind to misfolded proteins and either tag them for the ubiquitin/lysosomal degradation pathway or refold them into the correct structural configuration. Myocardial research has indicated gender bias in Hsp70 expression to be more abundant in men. It is hypothesized that an interaction with 17-beta estradiol (E2) with estrogen receptor-α (ER-α) and Hsp90 is decreasing the transcription of Hsp70. The premise behind this study was to examine this relationship, in addition to behavior, at the beginning of disease progression in a neurodegenerative transgenic mouse model of AD. Male and female 5xFAD transgenic mice at 4 months of age underwent a series of behavioral assays to assess motoric and cognitive abilities. These behavioral assessments included the use of open field, novel-object recognition and passive-avoidance tasks. Immunohistochemistry was utilized to assess the localization of Hsp70, 90, ER-α and Aβ. Western blot analysis was conducted to determine protein expression of Hsp70, 90, ER-α and Aβ. Preliminary results indicate that there is a gender-specific difference in performance in the passive avoidance task, with AD females exhibiting a significantly shorter latency to cross into the shock chamber compared to their AD male littermates. Preliminary results in histology and western blot analysis indicate possible differences in pathology related to certain protein expression as well. This study is the first to specifically examine the relationship between early pathology and behavior in 5xFAD mice from a gender-dependent perspective.

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Resting-State Functional Connectivity Measurement in the Mouse Brain using Photoacoustic Computed Tomography

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Abstract
Photoacoustic (PA) imaging is a non-invasive modality which combines ultrasonic resolution and optical contrast. This hybrid biomedical imaging modality allows structural, functional and molecular imaging. When a short and high energy pulsed laser light illuminates tissue, the optical absorber such as hemoglobin undergoes thermoelastic expansion, generating an acoustic pressure wave which is detected with an ultrasound transducer. In PACT (Photoacoustic Computed Tomography), ultrasonic transducers acquire photoacoustic signals, which are used as input to tomographic image reconstruction algorithm. In circular-view photoacoustic computed tomography, ultrasonic detection positions follow a ring while a pulsed laser beam is expanded to illuminate the tissue in the region of interest. In this study we have developed an affordable novel tomography system for small animal such as mouse or rat with a new mechanical/electrical design. Since our tomography system has capability of acquiring whole image in less than 1 second, we can monitor hemodynamic changes on animal’s brain for a long period of time and explore resting state functional connectivity in small animal’s brain. PACT for small animals is a prototype for making bed-side infant brain imaging system in clinics in the later studies.
Evaluation of 3,4-methylenedioxypropylvalerone (MDPV) and 4-methylmethcathinone (4-MMC) in rats trained to discriminate d-amphetamine.

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Recent reports on the abuse of novel synthetic cathinone derivatives (i.e. “bath salts”) call attention to serious public health risks of these chemicals. In response to this concern, a growing body of preclinical research has characterized the psychopharmacology and abuse liability of these substances, particularly that of two of the most common constituents, methyleneedioxypropylvalerone (MDPV) and 4-methylmethcathinone (4-MMC). The present study assessed the discriminative stimulus effects and pharmacodynamics of MDPV and 4-MMC in animals trained to discriminate d-amphetamine. Eight adult male Sprague-Dawley rats were trained to discriminate 0.5 mg/kg d-amphetamine (AMPH) from saline. Dose response curves were determined with AMPH (0.125 – 1.0 mg/kg), MDPV (0.125 – 2.0 mg/kg), and 4-MMC (0.25 – 2.0 mg/kg). Additionally, a range of selected doses of MDPV and 4-MMC were tested in combination with the D1 receptor antagonist SCH 39166 (0.3 mg/kg) to assess D1 receptor mediation in producing the interoceptive stimuli of these drugs. MDPV and 4-MMC both produced dose-dependent increases in drug-lever responding with full substitution for AMPH at 0.5 mg/kg MDPV and 2.0 mg/kg 4-MMC. SCH 39166 produced a marked downward shift in the MDPV dose response curve at all doses tested. Preliminary results indicate a similar downward shift with SCH 39166 in combination with 4-MMC. A comparison of the MDPV dose-response curve with that of 4-MMC suggests that MDPV has considerably more potent psychostimulant effects than 4-MMC. This is line with our previous findings indicating that MDPV and 4-MMC produce amphetamine-like and MDMA-like discriminative stimuli respectively. The downward shift in the MDPV and 4-MMC dose response curves produced by SCH 39166 implicates the involvement of D1 receptors in the discriminative stimulus effects of these synthetic cathinones. Additional receptor antagonist tests are currently in progress to further elucidate specific receptor mechanisms mediating the discriminative stimulus effects of MDPV and 4-MMC.
Eukaryotic translation initiation factor 4B modulates CGG repeat RAN translation in a Drosophila model of FXTAS

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder characterized by gait ataxia, intention tremor, and dementia. FXTAS results from a CGG repeat expansion in the 5'UTR of the Fragile X gene, FMR1. Repeat expansion leads to elevated CGG repeat-containing mRNA expression and an aberrant form of non-canonical repeat-associated non-ATG initiated protein translation (RAN translation) through the CGG repeats, producing a polyglycine containing peptide (FMRpolyG) that accumulates in patient neurons. How the translation of FMRpolyG peptide is regulated, however, is not well understood. Eukaryotic initiation factors (eIFs) are well-known to participate in canonical translation and modulate non-canonical initiation events. We screened a set of candidate eIFs to determine their roles in regulating CGG RAN translation in a Drosophila model of FXTAS. This screen identified the eIF4A DEAD-box helicase cofactor eIF4B as a significant modulator of CGG repeat associated toxicity in flies. EIF4B overexpression enhanced toxicity, while reduced eIF4B expression partially suppressed CGG repeat toxicity. Expression of eIF4B significantly increased FMRpolyG levels in flies but did not alter CGG repeat-mRNA expression, suggesting a selective enhancement of CGG RAN translation. As CGG RAN translation requires eIF4A and eIF4B enhances eIF4A activity, these results suggest a specific role for the eIF4A/B helicase complex in RAN translation and support a role for RAN translation in repeat mediated toxicity.
Cocaine and opioid pain relievers are highly abused drugs. Moreover, ~78% of cocaine and ~71% of opiate addicts are poly-drug users that also abuse other substances. Current treatments for addiction leave much to be desired as the majority of drug users relapse within the first year of treatment. The opioid system has emerged as a viable target for relapse prevention in drug addicts. For example, buprenorphine effectively decreases the number of positive drug tests in poly-drug users. Buprenorphine is a mu-opioid receptor (MOPr) and nociceptin receptor (NOPr) partial agonist, and a delta-opioid receptor (DOPr) and kappa-opioid receptor (KOPr) antagonist. Ultimately, there are concerns surrounding prolonged buprenorphine treatment due to physical dependence and addiction liability associated with MOPr agonists. To combat this issue, we developed BU10119 which has similar in vitro pharmacological properties to buprenorphine, but lacks agonist action at MOPr and has improved activity at NOPr. Here, we confirm that this in vitro pharmacological profile of BU10119 translates to in vivo behavioral assays in mice. BU10119 completely blocked the antinociceptive effects of the MOPr agonist morphine and KOPr agonist ethylketocyclazocine (EKC) in the warm water tail withdrawal assay, attenuated the antinociceptive effects of the DOPr agonist SNC80 in acid-stimulated stretching and caused a hyperalgesic response consistent with its NOPr agonism. A conditioned place preference mouse model of reinstatement was used to determine the ability of BU10119 to prevent drug seeking behavior. BU10119 dose dependently attenuated both cocaine- and stress-primed reinstatement, but did not alter cocaine-induced locomotor activity. The nonselective opioid antagonist naloxone, which has no pharmacological effects at NOPr, and the NOPr agonist SCH221510 were used for comparison to determine the receptor mechanisms involved in the anti-reinstatement effects of BU10119. When administered alone, naloxone and SCH221510 failed to attenuate cocaine-primed reinstatement. These results suggest that mixed efficacy activity at all opioid receptors (MOP, DOP, KOP, and NOP) might be necessary for the effects of BU10119 on cocaine seeking behaviors. This research was supported by R01DA007315 and T32DA007268.
Glioblastoma multiforme (GBM) is one of the most devastating forms of human brain cancer. Even with treatment patients are expected to only live a few months. In the Field Neurosciences Institute laboratory at CMU, great advances are being made utilizing the release of anti-inflammatory factors from mesenchymal stem cells (MSCs), for treating a variety of neurological diseases including GBM. However, while this treatment has significant potential for treating GBM, much work is needed to more effectively shrink the volume of the tumor. To address this need, this project was based on a premise that an ideal ratio of transplanted stem cells to tumor cells needs to be determined in order to improve treatment outcome. In order to better match MSCs to GBM cells, studies examining the growth kinetics of the GBM tumor cells within the animal brain are needed. To this end, the goal of this in vivo study, was to investigate the proliferation rate of the GBM cells using a nuclear marker bromodeoxyuridine (BrdU). GBM cells were first transplanted into 20 brains of rats that were divided in three groups and euthanized at different time point: one, two or three weeks after the GBM transplantation in the brain. Live images of tumor growth in vivo were taken at days 11, 17, 25 and 31 in a different set of animals; post-fixation immunohistochemistry was also completed for tumor growth. Results showed an increase in tumor size over the 3 weeks which was confirmed by live imaging. Ultimately, the results of this study will help to determine the optimal time for intervening with MSCs in order to reduce the proliferation of GBM cells in the brain.

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Title: The Development of Spatial Navigation Ability from Childhood to Adulthood

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Theme: Cognition and Behavior

Spatial navigation is a critical ability that allows humans to effectively reach target locations in their environment. Prior evidence shows age and sex differences in spatial navigation ability, with older participants requiring longer time, distance and more complex paths to reach their target. In the human brain, research in typically aging populations has found that smaller hippocampal volume is associated with more complex paths traveled to reach the target. However, the developmental trajectories of spatial navigation from childhood through adolescence and the underlying neural substrates, remain unclear. Additionally, the relationship between spatial memory and spatial navigation ability in children and adolescents is unclear.

We tested spatial navigation performance using a virtual Morris Water Maze task in 57 participants aged 5-21 years. Each participant completed 15 navigation trials, during which they used a joystick to reach a platform hidden under the water in the virtual swimming pool. To measure the spatial navigation ability, length and complexity or fractal dimensionality (FD) of the paths that participants traveled to reach the target were calculated. We found the average of the distance and FD values across all 15 trials and plotted them against trial number to determine improvement of spatial navigation ability across trials. Participants were then tested on their memory of the virtual environment using both free recall and object cued recall of the platform. The results from the spatial memory tasks were analyzed to see how spatial memory benefits spatial navigation. Additionally, we tested for the age and sex effects on distance and FD measures to determine differential developmental trajectories of spatial navigation between males and females.

We found an age-related decrease in path length. Males traveled less complex paths than females, indicating a better cognitive map. In males, better recall of the virtual environment was associated with a less complex path in younger participants; in contrast, in females, this association was observed in older participants. Overall, these findings showed an age-dependent increase of spatial navigation ability and also suggest a potential protracted maturation of spatial navigation ability in females compared to males.
Ablation of p75NTR expressing cells in the mouse olfactory bulb disrupts circuitry and anatomy

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The olfactory bulb (OB) receives synaptic input from olfactory sensory neurons and readily displays neuroplasticity following a loss of synaptic input. A loss of synaptic input has been shown to reduce tyrosine hydroxylase (TH) and dopamine (DA) in the OB. Similarly, activation of the p75NTR has also shown a reduction in TH. In the first experiment, we sought to determine if p75NTR is expressed by TH interglomerular neurons. ImageJ tissue analysis revealed that p75NTR and TH failed to colocalize on interglomerular neurons (n=3). In a second experiment, we sought to induce OB plasticity by ablating p75NTR expressing cells. Mice were immunolesioned with a p75NTR targeted saporin toxin and an untargeted control toxin. After six days of recovery, immunohistochemistry was performed to determine the expression and location of p75NTR within the OBs. We observed an average decrease of 65% in p75NTR signal for the dorsal region of the immunolesioned OB compared to the control OB (n=3). We also aimed to identify if GFAP signaling would be affected by immunolesion. Present data displays no significant change in GFAP labeling in the dorsal OB after immunolesioning (n=3). Ongoing studies are being performed to determine if p75NTR labeling is colocalized with glia cells and if the decreased p75NTR labeling correlates with decreased TH expression. Additionally, a loss of TH and DA is thought to increase OB sensitivity to incoming stimuli. The immediate early gene c-Fos can be used as an indicator of recent neuron activation. Studies are being performed to see if a measurable difference in the location and amount of c-Fos expression can be observed in mice either treated intranasally with a Triton X-100 solution or the p75NTR immunolesion. Triton X-100 treated mice have shown higher c-Fos expression per unit area relative to the PBS treated control mice. Data is currently being collected for c-Fos expression in p75NTR immunolesion mice. With these and future data, we can begin to provide a better explanation of p75NTR’s location and role in neural plasticity within OB circuits.
LASTING CHEMORECEPTION DEFICITS ARE OBSERVED IN CRAYFISH FOLLOWING AN ACUTE ATRAZINE EXPOSURE

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Crayfish are a keystone species because they play a crucial role in the aquatic habitat due to their ability to transfer energy within the benthic food web and between the benthic and terrestrial food chains. Environmental contaminants such as the commonly used herbicide atrazine, is known to have a negative impact on olfactory-mediated behaviors in aquatic animals. Because crayfish rely heavily on their chemosensory reception to obtain food and mates and because they live in turbid environments, negative impacts on chemoreception could negatively affect population size. Prior research in our lab has shown that an acute atrazine exposure has damaging effects on olfactory-mediated behavioral responses to both food and mate odors in the crayfish *O. rusticus*. What is not currently known is whether or not crayfish can recover any of their chemosensory abilities after this initial exposure or if the effects are long-term. Our research involved examining recovery of chemosensory abilities after exposure to sub-lethal, environmentally relevant concentrations of atrazine. Atrazine-treated crayfish (N=15) were exposed to 80 ppb (µg/L) atrazine for 96 hours. Control crayfish were exposed to water only for 96 hours. We then analyzed the ability of the crayfish to locate a food source using a Y-maze and fish flavored gelatin in one arm of the maze. A video tracking software was used to examine and compare time spent within 10 cm of the food source, time in correct arm of the maze, velocity, and time spent moving and not moving. We also examined total food consumed and total distance travelled in the tank. Next, we allowed the crayfish to recover for 24, 48 and 72 hours in fresh water. Every 24 hours, we re-examined the behavioral trials to determine if there were any changes in chemosensory-mediated behavior. Our data suggest that crayfish are not able to recover their chemosensory reception 72 hours post-atrazine exposure. For all times tested, control crayfish spend significantly more time in the arm with the food, near the odor source and consume more food than those crayfish exposed to atrazine. This is significant because a long-term reduction in chemosensory abilities can have lasting effects on foraging abilities and may thus impact population size.
The effect of ventral tegmental area Rictor knockout on susceptibility to chronic social defeat stress and stress-induced changes in morphine reward

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There is significant co-morbidity of addiction and depression and this may be due to dysregulation of common brain circuits. For example, similar alterations in signaling in the ventral tegmental area (VTA) have been identified in response to both chronic morphine and chronic social defeat stress (CSDS), a rodent model of depression. We are particularly interested in target of rapamycin complex 2 (TORC2) signaling in the VTA as it has been established to play a critical role in morphine reward and morphine induced neuroadaptations. However, whether it plays a similar role in stress-induced changes in morphine reward, or stress susceptibility itself, is unknown. To address these questions, we used viral and genetic approaches to decrease VTA TORC2 signaling by knocking out the expression of Rictor, a TORC2 component protein necessary for TORC2 function. Floxed-Rictor mice were either crossed with a TH-Cre mouse line to eliminate TORC2 signaling in all catecholaminergic neurons or underwent stereotaxic surgery to specifically decrease TORC2 signaling in the VTA via AAV-Cre infusion. The mice were then subjected to chronic physical or emotional CSDS and their susceptibility to stress was measured using the social interaction (SI) test. Following SI testing, mice underwent a two-bottle choice assay to assess voluntary morphine preference and consumption. In contrast to previous data that found that decreasing AKT signaling was sufficient to increase susceptibility to stress, we found that decreased VTA TORC2 signaling did not alter stress susceptibility. However, we did observe a difference in morphine consumption, as Rictor knockout mice (both TH-Cre positive and AAV-Cre) that underwent stress consumed significantly more morphine than stressed controls (intact TORC2 signaling). Additionally, following stress, Rictor knock-out mice maintained their increase in water and sucrose consumption, suggestive of an overall increase in consummative drive.

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This study will determine the effects of WIN 55, 212-2 administration on post-natal bone marrow derived MSC’s. MSC’s are multipotent stem cells which contain a high capacity for self-renewal and ability to differentiate. MSC’s have been shown to differentiate into bone, cartilage, muscle and fat cells. MSC’s have also been shown to differentiate into neuron-like cells. A unique aspect of MSC’s is their immunosuppressive effect; making them a viable option for transplantation. Previous research suggests that cannabinoid receptors are present in MSC’s and may influence cell proliferation, migration, differentiation and functional integration. The Cb1 and Cb2 receptors may play differential and potentially inverse roles in their effect on MSC’s. WIN 55, 212-2 is a potent full cannabinoid (Cb1) agonist. This study will evaluate whether WIN 55 administration influences the rate of MSC proliferation, migration and Cb1 receptor regulation. Cell proliferation will be determined based on 1μM and 10μM doses of WIN-55 dissolved in DMSO compared to a control group receiving only DMSO. Migration will be analyzed based on scratch analysis. Fluorescent staining for Cb1 will be conducted, as well as western blot analysis to quantify Cb1 receptors. The MSC’s have been verified based on morphology and fluorescence-activated cell sorting.
Ghrelin’s Influence on Neurogenesis and Neuroinflammation in Aged Rats

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This study will evaluate whether exogenous ghrelin administration attenuates hippocampal-related cognitive deficits in aged rats. Ghrelin is an orexigenic hormone secreted in the gastric fundus of the stomach during episodes of hunger and meal anticipation. Ghrelin readily crosses the blood brain barrier and is the ligand for the growth hormone secretagogue-receptor 1a (GHS-R1a). GHS-R1a receptors were originally identified in the hypothalamus and pituitary; regulating energy homeostasis. However, GHSR-1a receptors also proliferate in the dentate gyrus (DG) of the hippocampus, an area implicated in memory consolidation and spatial memory. In non-pathological ageing populations, hippocampal-related memory deficits often increase as a function of age. The slowing rate of adult neurogenesis in the subgranular zone (SGZ) of the DG, as well as increased chronic neuroinflammation, likely contributes to these deficits. Exacerbating the problem, serum ghrelin levels are inversely correlated with age in humans and rodents. Increasing serum ghrelin levels, exogenously or endogenously, reliably increase the proliferation and survival of neural progenitor cells in the SGZ. This study will determine whether osmotic mini-pump ghrelin administration increases neuronal proliferation and cell survival in the SGZ and granular layer (GL) of the DG. We will evaluate whether ghrelin plays an anti-inflammatory role in the hippocampus. This study will also determine whether ghrelin influences hippocampal-dependent behavioral measures using the Morris water maze task and radial arm maze task.
INCREASED RESPONSE TO EMOTIONAL STIMULI IN THOSE WITH MILD EMOTIONAL DISTRESS

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Several studies have shown that those who are depressed experience a higher level of shock/surprise when viewing positive stimuli, but there is variance in these reports. In the present study it was hypothesized that more emotionally distressed participants would have a higher skin conductance response (SCR) suggesting a greater emotional response when viewing positive images compared to those that are not depressed. The participants were given a modified Beck’s Depression Inventory (BDI) and were divided into 2 groups: control and those with possible mild-moderate emotional distress. None of the participants were considered to have possible mild, moderate, or severe depression. After baseline SCR was measured, the participants were shown 30 images containing positive, neutral, or negative images of people. Each image was shown for 5 seconds and SCR was measured. To understand each individual’s personal experience of each image, they rated the likeability of each image on a Likert Scale. Results showed that the individuals with mild emotional distress had an increased SCR than those in the control group ($p=0.024$). In future works, the International Affective Picture System will be utilized to assess SCR in sample with a more varied ranged of reported depression.

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Cortical fold opening mediates the effects of prenatal alcohol exposure on cognitive outcomes

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Introduction: Brain regional abnormalities in shape and cortical thickness have been observed in children with fetal alcohol spectrum disorders (FASD) using voxel-based morphometry and surface-based analysis. We have previously reported that prenatal alcohol exposure is also associated with a reduction in cortical folding complexity. We now examine the relation of this structural anomaly to cognitive function in these children and the degree to which it may mediate adverse effects of prenatal alcohol exposure on cognitive performance.

Methods: Participants were 24 9-year-old children with fetal alcohol spectrum disorders (9 fetal alcohol syndrome (FAS), 15 heavy exposed non-syndromal) and 16 age-matched controls from the Cape Town Longitudinal Cohort study. FAS was diagnosed by three expert dysmorphologists. Cortical gyrification was assessed by an automated method applied to 3D T1 weighted images and measured using global and regional sulcal indices and two region-based morphological measurements—mean sulcal depth and fold opening.

Results: Greater fold opening in several regions was associated with poorer performance on working memory, recognition memory, math, and cognitive flexibility. Cortical folding in several of these regions mediated the effect of prenatal alcohol exposure on these cognitive performance outcomes. For example, the addition of fold opening in the right parietal region in multiple regression models reduced the standardized regression coefficient for AA/day in relation to working memory from -0.42 to -0.05, $t=3.49$, $p=0.001$.

Conclusion: This study provides new evidence that reductions in cortical surface area may mediate adverse effects of prenatal alcohol exposure on cognitive function in childhood.

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4-methylmethcathinone (4-MMC) is a synthetic derivative of the CNS stimulant cathinone and a common constituent of illicit “bath salts”. Recreational use of this and other synthetic cathinones has recently increased in popularity as an alternative to other commonly abused psychostimulants, such as 3,4-methylenedioxymethamphetamine (MDMA) and cocaine. Neurochemical and electrophysiological studies indicate mephedrone is a substrate at monoamine transporters that increases extracellular dopamine and serotonin, similar to the amphetamines and MDMA. Consistent with its neurochemical profile, 4-MMC has a high abuse liability as indicated by evidence of self-administration by nonhumans. Furthermore, at high doses (10-30 mg/kg) 4-MMC produces locomotor sensitization and conditioned place preference in rodents. Although polysubstance use is common in recreational “bath salts” users, there is a paucity of preclinical research on the combined effects of synthetic cathinones and other psychostimulant drugs. As part of an ongoing effort to develop an animal model of polysubstance use, the current study assessed the effects of low dose mixtures of 4-MMC (1, 5 mg/kg) and cocaine (5 mg/kg) for conditioned place preference (CPP). Thirty-six adult male Sprague-Dawley rats were randomly assigned to one of six treatment groups that received injections of saline, 1 mg/kg 4-MMC, 5 mg/kg 4-MMC, 5 mg/kg cocaine, 5 mg/kg cocaine + 1 mg/kg 4-MMC, or 5 mg/kg cocaine + 5 mg/kg 4-MMC. Place preference was assessed in an unbiased design in which animals were randomly assigned to receive drug treatments paired with one compartment and saline injections paired with the opposite compartment of a two chamber place conditioning apparatus. The saline control group received saline injections paired with both compartments. Following a 15 min habituation trial, eight conditioning trials were conducted with four drug trials and four saline trials in alternating order with one trial per day for eight days. Activity and movement were recorded during conditioning trials. On the 10th day, animals were allowed to explore both compartments and time spent in each compartment was measured. Difference scores were calculated from the time spent in each compartment as an index of CPP. Statistical analysis indicated mixtures of 4-MMC and cocaine significantly increased activity, whereas the individual drugs produced minimal effects on activity. All drug-treated groups spent more time in the drug-paired compartment following conditioning, but differences from the saline control group were not statistically significant. The lack of statistical significance may be a consequence of the small sample size. Additional studies are ongoing to further evaluate the combined locomotor stimulant effects of 4-MMC with cocaine and other psychostimulants.
Substance use disorders are estimated to cost our country over $700 billion a year, and are a serious health concern, with relapse rates as high as 90%. Cues (e.g. people, places, paraphernalia) associated with the drug or drug-taking experience have been shown to be very powerful motivators that can result in drug-seeking behaviors, or relapse via Pavlovian-learning processes. However, there is individual variation in the extent to which a cue can attain such motivational value. The process by which cues can gain inordinate control over behavior is known as incentive salience attribution, and we have an animal model that allows us to study individual variation in the propensity to attribute incentive salience to reward-paired cues. In this model, sign-trackers (ST) are those rats that attribute incentive salience to a reward-predicting cue, and will approach and manipulate the cue itself during presentation; whereas goal-trackers (GT) assign only predictive value to the cue and go to the location of reward delivery upon cue presentation. Relative to GT, ST are also more impulsive, have higher cocaine break-point and are more susceptible to cue-induced reinstatement of drug-seeking behavior. The paraventricular nucleus of the thalamus is a brain region previously implicated in sign- and goal-tracking and has recently been shown to mediate drug-seeking behavior in various cocaine relapse models. The current study examined how individual variation in the motivational value of a drug cue was impacted by inactivation of the PVT during a test for cue-induced reinstatement of cocaine-seeking behavior. After separating rats into ST and GT, rats underwent 2-weeks of cocaine self-administration followed by a 2-week forced drug abstinence period. After the abstinence period several extinction sessions were conducted to extinguish the association between the context the rats previously received cocaine in, and the drug and drug cue. Rats then underwent a test for cue-induced reinstatement where drug seeking in response to the drug cue was measured. Before this test session rats received an infusion of either baclofen and muscimol (GABAB and GABAA agonists used to inactivate brain regions) or saline (control) into the PVT. Results show a significant interaction of drug-seeking behavior between phenotype (ST vs GT) and treatment (baclofen/ muscimol vs saline). Additionally, post-hoc comparisons showed ST that received saline treatment had greater drug-seeking behavior compared to GT that received saline treatment. There was also no difference in drug-seeking behavior between the ST PVT inactivation group, and the GT saline group. These results suggest that not only does the PVT mediate drug-seeking behavior in a test for cue-induced reinstatement, but that it differentially mediates this behavior depending upon individual variation in the motivational value of a drug cue.
PARKIN POSITIVELY MODULATES 26S PROTEASOME ACTIVITY IN THE STRIATUM

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The motor symptoms of Parkinson disease result from the chronic degeneration of nigrostriatal dopamine (NSDA) neurons. While NSDA neurons are susceptible, tuberoinfundibular dopamine (TIDA) neurons are spared. This pattern of DA neuronal susceptibility can be recapitulated with exposure to the neurotoxicant MPTP. Following exposure to MPTP, the recovery of DA neurons is dependent on the expression of parkin, which is increased in TIDA neurons, and decreased in NSDA neurons. Parkin is an E3 ligase, part of the ubiquitin proteasome system that targets proteins for degradation by the 26S proteasome. In addition, in vitro studies indicate that parkin can bind to the 26S proteasome and enhances its activity. Proteasome activity in the striatum (ST) of both wild-type (WT) and Park2$^{-/-}$ mice was measured following MPTP exposure. While MPTP exposure decreased proteasome activity in NSDA terminal regions of both WT and Park2$^{-/-}$ mice, proteasome activity was impaired in the TIDA terminal regions only in Park2$^{-/-}$ mice, indicating that the maintenance of proteasome activity is dependent on parkin expression. To test the hypothesis that parkin expression positively modulates the 26S proteasome, activity was assessed in vitro following exposure to MPTP or its active metabolite, MPP$^+$. Proteasome activity was decreased with increasing concentrations of MPP$^+$, but not with MPTP and the effect of MPP$^+$ on proteasome activity was abrogated by the addition of parkin, but not the control protein. Treatment of purified 26S proteasome with parkin resulted in increased proteasome activity compared to controls and when 26S proteasome was incubated with parkin followed by MPP+, proteasome activity was not significantly reduced. The role of parkin in promoting proteasome activity was further assessed in vivo using Park2$^{-/-}$ mice and rAAV2/5 parkin over expression. Proteasome activity was decreased in ST synaptosomes derived from Park2$^{-/-}$ mice compared to ST synaptosomes derived from WT mice. Treatment of WT mice with MPTP decreased parkin protein and proteasome activity in the ST synaptosomes. Proteasome activity in the Park2$^{-/-}$ mice treated with MPTP had a further decrease in proteasome activity compared to WT mice treated with MPTP indicating that parkin and MPP$^+$ have different mechanisms of action on the proteasome. Over expression of parkin via rAAV2/5 in the Park2$^{-/-}$ mice resulted in increased proteasome activity in ST synaptosomes. Collectively, these results indicate that parkin plays a role in positively modulating proteasome activity and protein homeostasis following acute neurotoxicant injury in DA neurons.
Mapping Prefrontal Cortex Contribution to the Development of Memory Formation

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The prefrontal cortex (PFC) is involved in memory formation; both activation and deactivation in this region support memory formation in adults. PFC shows protracted maturation and age-related increase in activation supporting memory formation. Little is known, however, about possible age effects in the magnitude of PFC deactivation, or the age effects in functional connectivity of PFC with other memory-related brain regions. We tested age effects in PFC activation and connectivity that supported subsequent memory of scenes in 83 participants (ages 8-25 years). Consistent with prior research, we found an age-related increase in subsequent memory activation within the dorsal lateral PFC. In addition, we found an age-related increase in subsequent memory deactivation in rostral lateral and superior regions of the PFC. Interestingly, individual differences in subsequent memory deactivation in the superior PFC mediated the age-related improvement in memory performance. We further investigated age effects in the functional connectivity patterns of PFC regions. The functional connectivity between dorsal lateral PFC and regions in the medial temporal lobe (MTL) was positive and increased with age, whereas the functional connectivity between the superior PFC and MTL was negative and increased with age, suggesting that an age-related increase in the level of anticorrelation between MTL and PFC supports improvement in memory functioning across age. Taken together, these findings demonstrate differential age effects in the contribution of PFC regions to memory formation and underscore the notion that protracted development of the PFC is a key factor in age-related increases in memory functioning from childhood to adulthood.
Title:
Trauma cue-potentiated fear behavior is blocked by post-traumatic administration of Vorinostat in rats.

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Abstract:
Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder characterized in part by hyperreactivity to trauma-related cues. This feature of the disorder, however, is often absent from animal models used to study maladaptations to traumatic stress exposure. To develop a model of hyperreactivity to trauma-related cues, we paired a scent cue with exposure to a traumatic stressor (the single prolonged stress, or SPS, model) and then investigated whether the scent cue itself could elicit fear reactions or enhance conditioned fear reactions at a later date. Additionally, we investigated whether giving the histone deacetylase (HDAC) inhibitor Vorinostat directly after SPS exposure would modulate this conditioning.

Thirty-two male Sprague-Dawley rats were used in this study, and were assigned to one of four groups: control/vehicle, control/Vorinostat, SPS/vehicle, or SPS/Vorinostat. All rats received exposure to a CS- scent (vanilla or lemon, balanced across groups) for 30 minutes. The next day, each rat was exposed to a CS+ scent (vanilla or lemon, balanced across groups) for 30 minutes prior to exposure to SPS or handling (control). SPS consisted of restraint for two hours, 20 minutes of forced group swim, a 15 minute rest period, and exposure to ether vapor until loss of consciousness (<5 minute). Immediately after regaining consciousness from ether vapor exposure, animals were intraperitoneally administered Vorinostat (50 mg/kg). After a 7 day incubation period, rats were tested for scent aversion using a two-chamber apparatus in which rats were free to choose between the chambers holding the CS- and CS+ scents. Finally, we determined whether scent cues could enhance fear behavior by exposing rats to contextual fear conditioning, three days of extinction, and reinstatement testing with the CS- and CS+.

We found that SPS-exposed rats did not show a conditioned aversion to the CS+ scent. All animals readily acquired conditioned fear responses, however control/Vorinostat, SPS/vehicle, and SPS/Vorinostat groups showed significantly less freezing during extinction than the control/vehicle group. When exposed to the CS+ scents in the context of fear conditioning, the SPS/vehicle group showed an increase in freezing relative to the last extinction session; this increase was not present in any other group, and was not present during exposure to the CS- scent. These results indicate that pairing of scent with SPS can produce a conditioned association that is capable of enhancing fear behavior even after a significant delay, and that inhibition of HDACs immediately following a traumatic stressor either prevents this association from forming or decreases its ability to enhance fear behavior. Future studies determining which subtypes of HDAC are responsible for this effect are important in guiding the development of pharmacological strategies to decrease the impact of trauma-associated fear memories while minimizing side effects.

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A novel method for measuring intrinsic efficacy of ligands at a G protein-coupled receptor
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Efficacy is the feature of a drug that determines the degree of its physiological effects. Thus, a reliable quantification of efficacy is required in order to make predictive correlations between in vitro activity and in vivo activity. Many problems exist with current methods for the calculation of efficacy, including variation between cell type, signaling output, and time-point of measurement. Here, we present a cell-free, signal transduction-independent method for the calculation of intrinsic efficacy of ligands that bind to the mu-opioid receptor (MOPr), a G protein-coupled receptor targeted by “orthosteric” agonists such as morphine and oxycodone. This technique uses a small camelid antibody, nanobody 39 (Nb39), as a biosensor to detect the formation of active-state MOPr resulting from ligand binding in real time. The binding of Nb39 correlates significantly with efficacy measured using more traditional techniques, but is much more sensitive and since it is measured in purified proteins it is not compromised by environmental factors. In addition, we show that positive allosteric modulators of MOPr (mu-PAMs), which bind to a site distinct from the orthosteric site, can also promote active-state formation of MOPr. This active-state formation correlates with the ability of the mu-PAMs to enhance the affinity of agonists in traditional binding assays. The technique can readily be applied to other G protein-coupled receptors to analyze the efficacy of both orthosteric and allosteric ligands.
Using single cell reverse transcription-polymerase chain reaction to distinguish direct- and indirect-pathway projection neurons of rat striatum
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The striatum integrates signals from numerous brain regions in order to influence a wide range of behavioral responses including decision-making and motivation. In addition, alterations in the function of striatal neurons play key roles in a number of diseases including drug addiction and obesity. The striatum itself is comprised predominantly of GABAergic medium spiny neurons (MSNs) that receive convergent dopamine and glutamate inputs. MSNs can be divided into two primary output pathways: MSNs containing D1 receptors and the neuropeptides prodynorphin and substance P that project directly to the basal ganglia (dMSNs), and MSNs containing D2 receptors and the neuropeptide proenkephalin that reach the basal ganglia indirectly via the globus pallidus external and subthalamic nucleus (iMSNs; see Yager et al., 2015 for review). It is generally accepted that these two pathways have opposing effects, with the dMSNs acting as a “go” signal to initiate behavior and iMSNs acting as a “brake” to inhibit behavior. Further, potentially addictive drugs and diet-induced obesity alter the function of MSNs, but relatively little is known about how these changes may differ in dMSN vs iMSNs. While dMSNs and iMSNs can be identified in reporter or transgenic mice, similar approaches are not available in rats. This is particularly problematic for whole-cell patch clamping, where traditional labeling/staining methods are difficult to use. Therefore, we have optimized a reverse transcription polymerase chain reaction (RT-PCR) protocol to identify dMSNs and iMSNs from single cells in rat striatum. After completion of whole-cell patch clamp recording experiments in adult striatum, the cell body contents were harvested into the recording pipette and this starting material was amplified using standard approaches. We designed primers targeted at rat cDNA for the D1R, D2R, prodynorphin, and proenkephalin genes for use with RT-PCR. As expected, we found that prodynorphin and proenkephalin were completely segregated in the majority of MSNs (~90%) and that MSNs expressing D1 also express prodynorphin. Thus, this procedure enables us to determine whether changes in MSN function identified with electrophysiological approaches occur to different degrees or preferentially in dMSNs vs iMSNs. This approach can be applied broadly, and is a useful tool for furthering our understanding of how alterations in these pathways contribute to addiction and motivation.
Determining the impact of Zika virus infection in mouse neural stem cells and mature neurons

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The recent emergence of Zika virus (ZIKV) has been linked to severe nervous system abnormalities in infants, such as microcephaly, and the autoimmune disorder, Guillain-Barré, in adults. The World Health Organization predicts that by the end of this year three to four million people will be infected with the Zika virus, a virus now known to be transmitted by mosquitoes and through sexual contact in humans. Very little is known regarding the basic biology of this virus and its implications in the developing brain, fueling the need to investigate the interactions of ZIKV with neural cells. So far, studies have shown that ZIKV affects the morphology and survival of neural stem cells and neural progenitor cells. In our study, we sought to assess the effect of ZIKV on neural stem cells (NSCs) and primary cortical neurons (PCNs) isolated from embryonic mouse brains using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and multielectrode arrays (MEAs), respectively. We were able to determine the impact of infection on the secreted proteome “secretome” of NSCs as well as how the infection impacts the neuronal firing capabilities of PCNs. These studies provide mechanistic detail on how ZIKV impacts the differentiation, survival, development, and function of neural stem cells and primary neuronal cultures.
SHORT-TERM SYNAPTIC PLASTICITY WITHIN RAT THALAMOCORTICAL CIRCUITRY

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It is well established that information transfer within thalamocortical circuits is dynamically regulated. Considering that tetanic activation of afferent pathways can lead to alterations in synaptic efficacy (i.e., long term potentiation, long term depression, and spike-timing dependent plasticity), we carried out a series of experiments to determine if similar forms of plasticity occur in thalamocortical circuits. Electrophysiological recordings were obtained from thalamocortical relay neurons in the ventrobasal nucleus (VB). The induction protocol involves tetanic electrical stimulation of corticothalamic afferents (5 pulses at 100Hz, repeated 400 times at 2Hz). This stimulation protocol produced a short-term (10-15 minutes) facilitation of the excitatory postsynaptic current (EPSC) amplitude in 31 of 42 neurons. Using paired-pulse stimulation (100 ms interval), the induction protocol produced a decrease in the paired-pulse ratio consistent with a presynaptic mechanism. Additional experiments indicate that the potentiation is calcium-dependent, since the enhancement was absent in calcium-free physiological solution. We also showed that the adenylyl cyclase pathway contributes to the train-induced potentiation. Activation of adenylyl cyclase by forskolin mimicked the potentiation and occluded the subsequent train-induced potentiation. Antagonizing adenylyl cyclase activity with the adenylyl cyclase inhibitor, SQ22536, significantly reduced the potentiation. The K⁺ channel blockers tetraethylammonium chloride (TEA) and 4-aminopyradine (4-AP) also attenuated the potentiation, indicating the role of K⁺ channels in the potentiation. These data suggest that tetanic activation of corticothalamic afferents activates the adenylyl cyclase pathway, leading to the closing of K⁺ channels, thereby depolarizing the axon terminals and inducing enhanced neurotransmitter release. This potentiation could provide a mechanism through which corticothalamic activity could impact and regulate information processing through thalamocortical circuits.
Loss of Specific Olfactory Sensory Neurons in Zebrafish After Chemical Exposure

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The zebrafish is an important model organism for studying vertebrate neural plasticity because of the natural turnover of olfactory sensory neurons (OSNs) and quick regeneration after damage. The three main types of fish OSNs are ciliated, microvillous, and crypt neurons that are distinct in structure and behavior. Ciliated OSNs detect bile salts that are important for social behaviors, microvillous OSNs detect amino acids that are important for feeding behaviors, and crypt neurons appear to mediate sexual cues. Previously, our lab has shown that chemical ablation of the olfactory organ using zinc sulfate results in the degeneration of ciliated sensory neurons 2 days after treatment along with the loss of bile salt detection, however, microvillous sensory neurons still remain as well as the ability to detect amino acids. This suggests that microvillous OSNs may be more resistant to damage in order to retain the ability to detect food stimuli. Our hypothesis is that neurons mediating reproductive and social behavior are more sensitive to damage while neurons required for food detection are more resistant. The purpose of this study is to show that after chemical treatment with Triton X-100 ciliated neurons are lost due to damage, but microvillous and crypt neurons remain. Adult zebrafish were intranasally infused with 0.7% Triton X-100 and were allowed to recover for 1 day, 2 days, and 5 days, post treatment. OSNs were identified using either anti-Hu (all OSNs), ant-TrPC2 (microvillous OSNs), anti Gasolf (ciliated OSNs), and anti-s100 (crypt OSNs). Comparisons in the amount of label were made between the treated side and the internal control side as well as with untreated control tissue. One day following Triton X-100 treatment there is a reduction in anti-Gasolf and anti-Hu labeling compared to the internal control side and untreated control fish. There were no differences noted in amount of anti-TrPC2 and anti-S100 label. This suggests that ciliated neurons are no longer present and there is an overall reduction of OSNs in the olfactory epithelium; however, microvillous and crypt sensory neurons remain after chemical exposure. This study provides a further investigation into the prospective resilience of microvillous sensory neurons and the retention of food sensing abilities after chemical damage. This work has relevance to general neuroprotective mechanisms that ensure proper functioning of sensory input after toxic insult.

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The effects of delta-opioid receptor ligands on the conditioned stimuli and rewarding effects associated with cocaine in rats

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Studies have shown the opioid receptor system modulates behaviors and neurocircuitry associated with reward and drugs of abuse. For example, delta opioid receptor (DOR) activation stimulates cocaine-seeking behavior in conditioned place preference (Kotlinska et al., 2010) and self-administration (Simmons and Self, 2009) studies. Further, DOR activation alone produces many stimulant-like properties, such as increased locomotor activity and generalization to cocaine and amphetamine discrimination; however, it fails to maintain self-administration behavior (Stevenson et al., 2005). To further probe the role of DORs in reward-related behavior, the current study investigates the effects of DOR agonists and antagonists on responding for presentation of cocaine-paired cues. Rats were trained to self-administer cocaine (0.56 mg/kg/injection) on a fixed ratio 1 schedule of reinforcement. Once cocaine-maintained responding was stable, responding for presentations of cocaine-paired cues in the absence of cocaine was evaluated following treatment with the DOR agonist SNC80 or the DOR antagonist naltrindole. After extinction, SNC80 dose-dependently increases responding for cocaine-paired cues without altering responses on inactive manipulandum. Conversely, the DOR antagonist naltrindole decreases responding for cocaine-paired cues, but only under some conditions. Overall, these data suggest the DOR system influences conditioned reinforcing effects of cocaine-paired stimuli and therefore may play a role in the development of cocaine addiction.
Effects of social isolation on behavior and prefrontal cortex morphology and neuronal activity

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The prefrontal cortex has been shown to participate in several roles in the regulation of social behavior. Dysfunction in the prefrontal cortex can lead to aberrant social behaviors similar to those found, for example, in autism. Critical periods of heightened plasticity are essential for normal development of the prefrontal cortex, and dysregulation during these developmental stages can result in a range of symptoms that correspond to neurodevelopmental disorders.

Socially isolated mice have been established as a model for autistic-like behaviors such as aberrant social interactions. Social isolation can also contribute to other behavioral dysfunction, including anxiety- or depressive-like behaviors, and has been demonstrated to alter the underlying neurological function in the prefrontal cortex. This suggests that social interaction with other animals contributes to the development and emergence of normal social behaviors in mice. Since development of the prefrontal cortex continues well into adolescence, it is likely that the social enrichment during this critical development stage is necessary for normalized social behavior. Correspondingly, lack of social enrichment during this critical period in adolescence likely contributes to the underdevelopment of the prefrontal cortex, giving rise to social dysfunction and aberrant social behavior. We hypothesize that social isolation during adolescence effects aberrant behavior in adult animals through changes in prefrontal neuronal morphology and electrical activity.

Male and female mice of strain C57BL/6 were weaned at 3 weeks of age and segregated into single housed or group housed cages. A minimum of six weeks later several tests were performed to assess for changes in behavior. Specifically, mice were tested behaviorally using the three chamber test to assess for social dysfunction, open field to assess any evidence of anxiety behaviors, and the forced swim test to assess depressive behaviors. These mice were then surgically implanted with recording electrodes to perform in vivo electrophysiology. A separate group of mice is being used to assess morphology of the cortical neurons in the prefrontal cortex. These mice express green fluorescent protein (GFP) in neurons of the cortical layer, allowing anatomical tracing of arborization by confocal microscopy and assessment of general morphology and number of neurons.

Together our studies will reveal whether behavioral changes caused by early social isolation correspond to changes in the activity or morphology of the prefrontal cortex.
Effects of intravenous injection of a corticotropin-releasing factor receptor-1 antagonist on anxiety-like behavior in the rat.

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**Background:** Corticotropin-releasing factor (CRF) is secreted by the paraventricular nucleus of the hypothalamus as part of the stress response, and has effects in the brain including the amygdala. CP 154,526 (CP), a CRF-1 receptor antagonist, has been shown to modulate anxiety-like behavior when administered systemically. This pilot study aimed to determine the effects of intravenous (IV) injection of CP on anxiety-like behavior and to determine an anxiolytic dose for use in future studies.

**Methods:** Twenty-four male rats assigned to one of three groups (n=8/group), including vehicle, 2 mg/kg CP, or 6 mg/kg CP, were given tail-vein IV injections and subjected to a battery of tests to assess anxiety-like behavior. First, the animals were tested in an open-field (OF) box and then immediately placed in a glass cylinder to record grooming behavior. Finally, the animals were evaluated using an elevated plus maze (EPM). EPM and OF behavior were recorded and analyzed using Ethovision software, and grooming was digitally recorded and scored manually. Data were analyzed using one-way ANOVAs.

**Results:** IV-administration of CP produced trends in anxiety-like behaviors in all 3 tests, but none reached significance (OF (Fig. 1), EPM, & grooming p= 0.125, 0.227, & 0.712, respectively).

**Conclusion:** This pilot study suggests that IV-administration of a CRF receptor-1 antagonist may produce anxiolytic effects, particularly at 6 mg/kg CP. Additional animals per group are necessary to clarify our findings.

**Funding Source:** Funding was provided by Wayne State University Department of Psychiatry and Behavioral Neurosciences.
Macrophage-Dependent Impairment of α2 Autoreceptor Inhibition of Ca\textsuperscript{2+} Currents in Sympathetic Neurons Contributes to Hypertension in DOCA-Salt Rats

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Norepinephrine (NE) release from perivascular sympathetic nerves is regulated by prejunctional alpha-2 autoreceptors (α2AR), which inhibit nerve terminal N-type Ca\textsuperscript{2+} channels. We showed previously that macrophage infiltration and superoxide production in mesenteric arteries (MA) impairs α2AR-mediated inhibition of NE release. We tested the hypothesis that macrophages disrupt α2AR coupling to N-type Ca\textsuperscript{2+} channels in sympathetic neurons.

**Methods:** The DOCA-salt rat model of hypertension was used for this study. Immunohistochemistry was used to localize macrophages in MA. Ca\textsuperscript{2+} current inhibition was evaluated using whole-cell patch clamp on cultured sympathetic neurons of the celiac ganglion. Liposome-encapsulated clodronate (LEC) was used to deplete macrophages.

**Results:** Macrophage infiltration is increased in MA of DOCA-salt hypertensive rats. The α2AR agonists NE and UK14304 inhibit Ca\textsuperscript{2+} currents in neurons from normotensive but not hypertensive rats. In all neurons, yohimbine (α2AR antagonist) attenuates the effects of NE and dialysis of GppNHp (non-hydrolyzable GTP analog) inhibits Ca\textsuperscript{2+} current equally. In DOCA-salt rats, LEC treatment prevents macrophage infiltration in MA, reduces blood pressure elevation, and preserves α2AR-mediated inhibition of Ca\textsuperscript{2+} current in sympathetic neurons.

**Conclusions:** In DOCA-salt hypertensive rats, α2AR dysfunction leads to impaired modulation of Ca\textsuperscript{2+} current that is independent of factors downstream of receptor activation. Macrophage depletion preserves α2AR function and protects against blood pressure elevation.

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Understanding the role of giant ankyrin-G in GABAergic synaptogenesis
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Bipolar disorder is a common, chronic mental illness characterized by pathological swings in mood ranging from mania to depression. Available therapeutics are insufficient for effectively treating individuals with bipolar disorder, thus there is an unmet need to identify the genetic and molecular mechanisms that underlie this disease. Several large genome-wide association studies have identified ANK3 as the most consistent and significant gene associated with bipolar disorder. The giant, 480kDa ankyrin-G (product of the ANK3 gene) is a critical adaptor protein that organizes neuronal domains essential for the proper formation of axon initial segments, nodes of Ranvier, and GABAergic synapses. GABAergic inhibitory signaling is essential for the proper synchronization and function of neuronal networks that underlie cognition, mood, and behavior and abnormalities in GABAergic interneuron circuitry have been linked to bipolar disorder. Although the 480kDa splice variant of ankyrin-G is a key regulator of GABAergic synapse formation, there is a clear lack of understanding regarding the exact mechanisms by which ankyrin-G controls GABAergic circuit development. Using cultured neurons, we have shown that giant ankyrin-G interacts directly with GABA receptor associated protein (GABARAP) to inhibit GABA_A receptor endocytosis and stabilize GABAergic synapses. To test this mechanism in vivo, we generated a mouse model with a W1989R mutation in Ank3, which has been shown to completely abolish both ankyrin-G association with GABARAP, as well as GABA_A receptor clustering. Interestingly, preliminary data from coronal brain sections from Ank3 W1989R mice showed the loss of parvalbumin-positive GABAergic basket cell synapses on the cell body of cortical pyramidal neurons. In addition, these mice exhibited a decrease in parvalbumin expression in interneurons, consistent with phenotypes seen in bipolar patients. As was seen in vitro, axon initial segments and nodes of Ranvier are spared in the W1989R mouse, making this a useful model to study the specific role of basket cell circuitry in normal neuronal activity and how dysfunction in these circuits might contribute to neuropsychiatric disease.
Current methods used to treat spinal cord injury with stimulation include functional electrical stimulation and intrathecal electrical stimulation. Both of these methods offer quality of life improvements for patients but are not entirely effective. Both of these methods have the drawback of rapidly fatiguing the stimulated motor units. Optogenetic stimulation provides a method of stimulation that can be done at the neuronal level and does not result in the same rapid fatigue as electrical stimulation. The current drawback of traditional optogenetic stimulation is the invasiveness, as this approach requires an implanted light source.

Here we describe a method for the activation of optogenetic constructs in a spinal cord injury model where genetically expressed opsins are activated by a light-producing luciferase that is fused to the opsin (luminopsin). Using this approach, cells expressing the construct are activated by the injection of the luciferase substrate allowing for light to be produced by the luciferase. The light sensitive opsin then opens, allowing for non-selective cation flow, exciting the neuron. This new approach can be applied to distinct populations of endogenous neurons and to neuronal progenitor cells for the direct stimulation of the grafted cells.
Split luciferase based genetically encoded calcium indicator

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Physiological roles of calcium are wide and varied: for example, calcium is required for the release of synaptic vesicles which helps in the propagation of neural impulses. Hence, the detection/imaging of calcium in live cells provides useful insight about cellular physiology. Genetically encoded calcium indicators (GECIs) coupled with fluorescent proteins have been employed to achieve successful calcium detection. In GECIs, the calmodulin-M13 protein complex is employed as a calcium sensing unit which undergoes a conformational change in the presence of calcium, a contraction to be exact. This contraction leads to a shift in wavelength of the fluorescent protein resulting in enhanced fluorescence. However, fluorescence-based GECIs have their own set of disadvantages such as they require an external, invasive light delivery for excitation. There is also the concern of photo-bleaching, as is true for all fluorescent based indicators.

We have employed the same calcium sensing calmodulin-M13 protein complex, but switched out the fluorescent reporter with a luciferase enzyme reporter which is split in half. A mutated form of *Gaussia* luciferase known as slow burn *Gaussia* luciferase (sbGluc) served as the reporter. The two halves of the luciferase are separated by the calmodulin-M13 protein complex. Thus we were able to inactivate the enzyme in the absence of calcium (since the two halves are separated). The influx of calcium into the cell contracts the calmodulin-M13 complex and brings the two halves of the luciferase closer to form an active enzyme, which in the presence of its substrate (coelenterazine or CTZ) gives off light. We call this split luciferase based GECI LuminCaMPsin4 or LMC4.

We are currently creating versions of LMC4 that are localized at different cellular organelles (endoplasmic reticulum, Golgi apparatus and mitochondria) and anchored to the plasma membrane. We will present data on several of these versions.
A SURVEY OF THE EFFICACY OF FIVE ANXIOLYTIC MEDICATIONS ON C57BL/6 MICE

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C57BL/6 mice are a common inbred strain of mice used to produce transgenic animals that will be used for behavioral experiments aimed at assessing anxiety and fear. However, the use of this strain for these types of experiments has been questioned due to their low level of baseline anxiety-like behavior and their lack of response to several anxiolytic medications, such as chlordiazepoxide, diazepam and buspirone. In this study, we measured anxiety-like and fear phenotypes of C57BL/6 mice that were treated with serotonin modulating drugs in order to address the validity of using this particular strain of mice as a genetic background for the development of transgenic animals that will be used to address anxiety and fear. We tested the efficacy of the selective serotonin reuptake inhibitors (SSRIs) fluoxetine (FLX,) and citalopram (CIT), the selective serotonin and norepinephrine reuptake inhibitor (SSNRI) venlafaxine (VLF), the serotonin modulator and stimulator (SMS) vortioxetine (VTX) and the noradrenergic and specific serotonergic antidepressant (NaSSA) miratzapine (MTZ) on anxiety-like behaviors and fear measures in C57BL/6 mice, including; open field, light/dark box, zero maze, and Pavlovian fear conditioning. We also tested all of the groups in marble burying, as that is a standard task used to test the efficacy of depression and anxiety medications in rodents. As a control to ensure that our method of drug delivery was valid, we tested marble burying, open field and Pavlovian fear conditioning on BALB/C mice treated with FLX and VLF, as they are known to respond favorably to commonly used serotonin modulators. In C57BL/6 mice, FLX, CIT and VLF treatment all resulted in a reduction in the number of marbles buried in the marble burying task, confirming bioavailability at an effective dose, as well as suggesting a decrease obsessive compulsive-type behavior. VTX and MTZ did not have a significant effect. Both FLX and VLF caused a reduction in marbles buried in BALB/C mice. In the open field task, all of the C57BL/6 groups treated with medication travelled significantly less total distance than the control group, with VLF, VTX and MTZ treatment resulting in an increase in the percentage of total distance travelled in the center zone. FLX and CIT did not have a significant effect on the percentage of distance travelled in the center. In BALB/C mice, only VLF treatment resulted in a decrease in total distance travelled, with both FLX and VLF treatment resulting in an increase in percentage of total distance travelled in the center zone. In the light/dark box task, FLX and VLF resulted in a decrease in the amount of time (seconds) spent in the light chamber, compared to the control group, suggesting that these two drugs cause an increase in anxiety-like behavior in C57BL/6 mice. None of the drugs increased the amount of time spent in the light zone, which is typically taken as a decrease in anxiety-like behavior. FLX treatment also resulted in a decrease in the distance travelled on the open arms of the zero maze in C57BL/6 mice, again, suggesting an increase in anxiety-like behavior. None of the drug treatments resulted in an increase in the distance travelled on the open arms of the zero maze. All groups (both C57BL/6 and BALB/C mice) were subjected to Pavlovian fear conditioning. After three days of training, C57BL/6 mice treated with MTZ and VTX showed an increase in freezing behavior, indicating an increase in fear or anxiety-like behavior, compared to the control group. None of the treatments resulted in a decrease in freezing behavior, which was the expected result. In BALB/C mice, both FLX and VLF treatment resulted in a decrease in freezing behavior after three days of training, indicating a decrease in fear or anxiety-like behavior. This study suggests that C57BL/6 mice are not an appropriate genetic background for transgenic animals that are to be used to study anxiety and fear, as they do not show a decrease in anxiety-like behavior in light/dark box or zero maze, or fear in response several serotonin modulating drugs, as well as others presented in the literature. In fact, this strain of mice exhibits an increase in anxiety-like behavior and fear in response to multiple treatments. This is an interesting result, as 15%-40% of individuals with anxiety either do not respond to treatment, or experience an increase of their symptoms while being medicated, with no known explanation. Thus we suggest that C57BL/6 mice could serve as a model to study the neurobiological substrates of treatment-resistant anxiety.
FosB and ΔFosB, Potential Regulators of Parkin in Tuberoinfundibular Dopamine Neurons

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A hallmark of Parkinson disease (PD) is the progressive loss of nigrostriatal dopamine (NSDA) neurons. In mice, these neurons are preferentially damaged through exposure to the neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Another population of DA neurons that respond to MPTP, but are able to recover are the tuberoinfundibular DA (TIDA) neurons. This recovery is correlated with an increase in parkin mRNA and protein. Parkin is a product of the PARK2 gene, which is linked to autosomal recessive or juvenile PD. Parkin has multiple functions in neurons and is predicted to protect against the neurotoxic effects of MPTP. Differential expression of transcription factors that regulate the parkin promoter could explain the differential susceptibility of DA neurons. The parkin promoter is bidirectional consisting of an approximately 200 bp segment of DNA that regulates the expression of the PARK2 gene as well as parkin co-regulated gene. Potential transcription factors of parkin were identified using TFSEARCH, PROMO, and Patch 1.0. Activator Protein 1 (AP-1), which is a heterodimer composed of proteins from the Fos, Jun, and ATF families, was one of the predicted transcription factors of parkin. In the present study, the temporal expression and cellular localization of FosB and ΔFosB after acute neurotoxicant administration were examined. Male C57BL/6j were injected with 20 mg/kg MPTP (sc) and decapitated at 1, 2, 4, 6, 8, 12, or 24 h post-injection. Control mice were injected with saline (10 mL/kg; sc) and killed 24 later. Brains were frozen, sectioned, regions containing the cell bodies of the TIDA (arcuate nucleus; ARC) and NSDA (substantia nigra; SN) neurons were dissected and processed for Western blot analysis. The results reveal that expression of FosB and ΔFosB correlates with parkin, increasing in the ARC and not in the SN. As ΔFosB is a highly stable truncated form of FosB, both FosB/ΔFosB and parkin were measured 1, 3, and 7 days after a single injection of 20 mg/kg MPTP (sc). Elevated ΔFosB and parkin protein levels were observed over all days in the ARC, but did not change in the SN. Furthermore, total FosB protein was localized to nuclei of NSDA and TIDA neurons, and expression of each FosB and ΔFosB examined in cytoplasmic and nuclear fractions derived from the ARC and SN. Though the number of DA neurons expressing total FosB does not change at 6 h post-MPTP, ΔFosB does increase in the nuclear fraction from the ARC. These results reveal that expression of FosB and ΔFosB correlate with the differential expression of parkin, increase prior to parkin, and are predicted to bind the parkin promoter sequence, which suggests that FosB and ΔFosB may act to regulate parkin expression in response to neurotoxicant exposure.
The enteric nervous system (ENS) controls gastrointestinal (GI) motility. The peristaltic reflex is a propulsive motility pattern that requires coordinated contraction and relaxation of the GI smooth muscle. The current model of the peristaltic reflex circuit has one class of inhibitory motor neuron that co-releases nitric oxide (NO) and ATP. NO and ATP mediate slow and fast inhibitory junction potentials (IJPs) and smooth muscle relaxations, respectively. NO and ATP release are dependent on voltage gated Ca\(^{2+}\) channel (VGCC) activation. However, specific subtypes of VGCCs may be involved in driving NO and ATP release. We used immunohistochemical methods to localize nitric oxide synthase (NOS) and the vesicular nucleotide transporter (VNUT) in myenteric neurons of the mouse colon. We also nerve-evoked mechanical responses of colon circular muscle rings in vitro using tissues from wild type and P/Q-type VGCC deficient mice (tottering, tg/tg). We found NOS+ nerve cell bodies in myenteric ganglia and some nerve fibers that co-expressed NOS and VNUT immunoreactivity. We also found nerve fibers supplying the muscle that expressed VNUT- but not NOS-immunoreactivity. Neurogenic relaxations of the circular muscle were inhibited by tetrodotoxin and by CdCl2, indicating that the relaxations were nerve mediated and dependent on voltage-gated Ca\(^{2+}\) channels. Neurogenic relaxations were similar in tissues from wild type and tg/tg mice. Our results showed that P/Q type dysfunction does alter inhibitory neuromuscular transmission in the mouse colon. There may also be multiple populations of inhibitory motorneurons supplying the muscle layers (NOS neurons, VNUT neurons and NOS/VNUT neurons). These studies highlight the complexity of the enteric nervous system. Our results also indicate that there is a high degree of functional redundancy that may ensure that propulsive motility patterns are sustained even when there may be defects in one type of inhibitory motorneurons (Supported by 1R01DK094932)
Different sources of neural stem cells have similar morphological phenotypes but differences in spontaneous spike activity.

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Neural stem cells are characterized as self-renewing cell populations with the ability to differentiate into the multiple tissue types of the central nervous system (CNS). These cells can differentiate into mature neurons, astrocytes, and oligodendrocytes. This category of stem cells has been shown to be an effective treatment for neurodegenerative diseases such as Parkinson’s disease and following traumatic injury to the CNS such as traumatic brain injury, stroke, and spinal cord injury. Most treatment studies with neural stem cells in animal models use embryonic brain derived neural stem cells. This approach presents both ethical and feasibility issues for translation to human patients. Adult sources of tissue are a more practical source of stem cells for transplantation therapies in humans.

Some adult tissues such as bone marrow and adipose tissue contain discrete populations of multipotent and embryonic like stem cells. Of these stem cell populations, some are able to respond to neuronal growth factors and can be expanded in vitro, forming neurospheres analogous to cells harvested from embryonic brain tissue.

Here, we describe a method for the collection and culture of adipose tissue which results in a population of neural stem cells that are able to be expanded in vitro and be differentiated into neuronal-like cells. These adipose derived cells display a similar phenotype to those derived from embryonic sources. When differentiated into neurons, cells derived from adipose tissue show spontaneous spike activity which is similar in waveform to the embryonic brain derived cell lines but show higher frequencies of spiking and bursting activity.
Determining Specific Nicotinic ACh Receptor(s) Activation Following Enhanced Release of ACh in a ‘Mixed’ Retinal Culture System: Use of Selective Positive Allosteric Modulators.

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Glaucoma, a neurodegenerative disease, is a leading cause of blindness. It is associated with increased intraocular pressure that may lead to death of retinal ganglion cells. In vitro and in vivo evidence suggests activation of acetylcholine (ACh) receptors can have neuroprotective effects against this neurodegeneration. We have been examining whether a potential Alzheimer’s drug (DMP 543) can increase ACh release in the retina, as in the brain, to activate these protective receptors. Previous experiments involved labeling retinal ACh in an ‘eye-cup’ preparation with tritium and measuring release with a liquid scintillation counter. DMP 543 was found to have a dose-dependent effect on the basal release of ACh from the pig retina. Other ACh release experiments included determining the dose-dependent enhancement of a standard depolarizing pulse of potassium. More recently we have been conducting experiments with a dissociated, ‘mixed’ retinal cell culture system presumed to contain cholinergic amacrine cells and retinal ganglion cells (RGCs). This is in contrast to an isolated RGC culture system using a ‘panning’ procedure, we have used previously to examine direct effects on RGCs. The addition of DMP 543 was found to have a dose-dependent effect on the survival of cells in our ‘mixed’ culture system. This indicated the presence of cholinergic cells which could be induced with DMP 543 to increase the release of ACh, and thereby increase the survival of retinal neurons. Currently, we are attempting to determine the identity of which nicotinic ACh receptor(s) (nAChR) are involved in this increased survival. Our experimental approach has involved the use of ‘positive allosteric modulators’ (PAMs). PAMs will not activate a ‘silent’ receptor, but will enhance the response after that receptor is activated. We have focused our investigations on PAMs selective for the alpha7 nAChR (using PNU 120596) and the alpha4beta2 nAChR (using NS 9283). Cultured ‘mixed’ retinal neurons were either unexposed (used as controls), exposed to DMP 543 alone, exposed to one of the selective nAChR PAMs alone, or to DMP 543 and one of the PAMs together. These experiments should allow us to determine which nAChR dominates in the increased survival after exposure to DMP 543, and possibly basal activation of the different nAChRs in the absence of DMP 543.
AGING CONVERNS A RELATIVE RESISTANCE TO VIRAL VECTOR-MEDIATED GENE DELIVERY IN THE RAT NIGROSTRIATAL AND STRIATONIGRAL SYSTEM

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Clinical trials currently examine the efficacy of viral vector-mediated gene delivery for treating age-related neurodegenerative diseases such as Parkinson’s disease (PD). While viral vector strategies have been successful in preclinical studies, to date, human clinical trials have disappointed. This may be partially due to the fact that preclinical studies fail to account for aging as an important covariate despite the fact that aging is the primary risk factor for PD. Previously, we found that gene transfer utilizing recombinant adeno-associated virus serotype 2/5 (rAAV2/5) results in decreased transduction efficiency in the aged (20 month) rat nigrostriatal system as compared to the young adult (3 month) rat (Polinski et al., 2015). In the present series of experiments, we investigated whether the phenomenon of deficient transduction in the aged nigrostriatal system is generalizable to other vector constructs and circuitry. To accomplish this, we compared the transduction efficiency of rAAV2/5, rAAV2/2, rAAV2/9, or lentivirus (LV) expressing GFP in young (3 month) or aged (20 month) male Fischer344 rats following injections into either the substantia nigra (SN) or the striatum, the structure most often targeted in PD gene therapy clinical trials. Four weeks after injection in the SN, three of the four vector constructs (rAAV2/5, rAAV2/2, and LV) were less efficient in transduction of the aged nigrostriatal system, whereas rAAV2/9 GFP was equally as efficient in young adult and aged rats. Furthermore, four weeks following injection into the striatum, all three rAAV serotypes were deficient in transducing the striatonigral system whereas LV was equally as efficient between ages. In addition, we examined the effect of age on transduction by a clinically-relevant construct, rAAV2/2 expressing glial cell line-derived neurotrophic factor (rAAV2/2 GDNF). This exact approach is currently being used in a clinical trial for PD (NCT01621581). One month post-striatal injection, aged rats exhibited significantly less GDNF in the aged striatum as well as anterograde structures (pallidus, and entopeduncular nucleus). Experiments examining activation of the downstream GDNF pro-survival signaling cascade in young adult and aged rats and the mechanism of the age-related deficiency are ongoing. These results demonstrate that the aged nigrostriatal and striatonigral systems are less amenable in general to viral vector-mediated gene transfer. An understanding of how age impacts viral vector transduction efficiency and identification of the mechanism of reduced transduction efficiency will be required for efficient protein delivery in future gene therapy clinical trials for age-related neurodegenerative diseases.

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Mental representations of visual stimuli contain both perceptual information (e.g. color, shape and size) and semantic information (e.g. the meaning of the stimulus). In our previous studies, we found that adults (18-57 years old) substantially relied on semantic information in recognition test of rapidly presented images. We also found that a longer interval between two stimuli while encoding increases recognition in the long term but has no significant effect in the short term. It would be of theoretical importance to examine whether this pattern is true in children (8-17 years old), given that the neural correlates that support visual perception, semantic encoding and recognition memory are still developing from childhood to early adulthood.

To further investigate this pattern, we used a novel Rapid Serial Visual Presentation (RSVP) task, which measures the recognition of rapidly presented images in procession, while manipulating test time (i.e., immediate vs. delayed), inter-stimulus interval (ISI) (i.e., 0 millisecond or 800 milliseconds), and image type (i.e., previously presented target images, semantically similar decoy images or semantically dissimilar non-decoy images). We assessed reliance on semantic information in recognition by assuming that the false recognition of a decoy indicates that the response was made based on semantic information. Seventy-two healthy individuals of two age groups (i.e., 8-17 years old vs 18-57 years old) were tested in this study. A main effect of age was observed, with adults being better than children in recognition performance. Interestingly we found that across the two age groups, ISI had little impact on semantic information reliance in the immediate recognition test. However, in both age groups, ISI did have a significant impact in the delayed recognition test in that stimuli presented with a 800ms ISI were recognized better than those presented with a 0ms ISI. Overall, our preliminary findings suggest that there may not be a significant difference between children and adults in the extent of semantic information reliance and the effects of ISI and test time on visual recognition; i.e., the patterns in visual recognition observed in adults are likely maintained from childhood.
ESTIMATING FUNCTIONAL CONNECTIONS AND INTRINSIC DYNAMICS FROM HIGH-RESOLUTION CORTICAL ACTIVITY OPTICAL IMAGING DATA

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The typical dynamics of cortical activity are largely unknown. In principle, voltage imaging methods have the resolution to characterize these dynamics, but at the moment technical issues such as noise hinder this goal. We have developed methods to reduce the noise and artifacts in this rapid imaging modality. Then we fit a high-dimensional model to high-resolution voltage imaging data in lightly anesthetized (0.5% isoflurane) mouse cortex. The model includes intrinsic regional oscillations, thalamic input, and communication between cortical regions. We estimate the parameters by a combination of constrained regression and Sliced Inverse Regression to high-resolution time series of cortical activity over most of one hemisphere of mouse cortex, obtained by voltage-sensitive dye imaging at 150 Hz. We estimate the relative contributions of each of the major components of the model to the fluctuations, and then estimate the effective connectivity between different cortical regions under these conditions. Our work suggests one path toward integrating models and data through the new technologies that are being developed under the BRAIN initiative.
Increase in microglial activation genes’ expression is concurrent with synaptic genes’ downregulation in the context of schizophrenia

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Two of the salient observations in schizophrenia pathology are dysregulated inflammatory markers and synaptic loss within the brain. Microglia have become targets of schizophrenia research due to their well-established regulatory roles in both inflammation and synaptic regulation. Particularly during high inflammation, microglia increase synaptic pruning through their phagocytic mechanisms. Additionally, some of the most common antipsychotics used to treat schizophrenia have had some evidences of downregulation of microglial activity in cell culture studies. What has not been investigated is whether human gene expression data in vivo can illustrate that microglial activation is concurrent with synaptic loss overall and particularly whether this occurs in a more exaggerated fashion in schizophrenia. Because any one gene might be expressed by multiple cell types and regulated by a number of different cellular processes, we propose using a robust composite score of genes that are expressed during microglial activation. We used previously published Affymetrix microarray data from treated cell culture models from Martinez et al* to determine genes that are differentially expressed in microglia in their respective activation states. Preliminary results show that the gene set we identified as associated with microglial activation show a strong negative correlation to the composite expression of GABA receptor genes. This was also demonstrated with both glutamate receptor and neurexin gene family composite expressions. These methods could be useful for identifying potential therapeutic targets in microglial activation in schizophrenia as well as describing the microglial cell proportions and microglial activation within other datasets of neurological disease states.

FGF-dependent mechanism of otic induction in derivation of inner ear organoids from Pax2EGFP/+ mouse embryonic stem cells

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Stem cells represent a potential source of cell types that are difficult to obtain for study in the lab. The sensory hair cells of the inner ear present a particular challenge: they are relatively scarce and concealed by the temporal bone. Therefore, a major goal of hearing research is the efficient production of hair cells for downstream applications such as replacement therapy, developmental studies, and drug discovery. Recently, a method for producing inner ear organoids from aggregates of mouse embryonic stem (ES) cells was established (Koehler et al., 2013). This method recapitulates basic signaling events of otic development, including activation of fibroblast growth factor (FGF) receptors during otic induction. A small percentage of cells then upregulate Pax2 and commit to otic fate. In chicken and zebrafish development, otic induction is mediated by the ERK pathway downstream of FGF receptors (Wang et al., 2015; Yang et al., 2013). However, the mechanism has not been established in mammals, and FGFs are known to signal through additional pathways including AKT and PLCγ. Elucidating this mechanism may facilitate efficient yields of hair cells for practical use. To study otic induction in a mammalian model, we adopted the inner ear organoid method. We used Pax2EGFP/+ mouse ES cells with EGFP inserted upstream of one Pax2 allele. This provided a report of Pax2 upregulation during FGF-driven otic induction. FGF2 was applied at 0, 5, 25, and 100 ng/mL, and ERK phosphorylation was assayed in some aggregates via Western blotting. Other aggregates were observed over several days as they developed internal EGFP+ otic vesicle-like structures, which pushed outward and expanded into large protrusions. EGFP+ cells became concentrated at the organoid regions (i.e., the protrusionaggregate borders). Organoids were fixed with 4% PFA and stained by immunofluorescence to confirm presence of hair cell-like cells at day 20. Finally, the effect of Pax2 dose on organoid formation was examined using Pax2EGFP/EGFP mouse ES cells, which express a lower amount of Pax2. The degree of ERK phosphorylation increased with FGF2 dose within 3 hours of application. In addition, the formation of organoids correlated positively with both FGF2 and Pax2 dose. Organoids contained Myo7a+ cells with F-actin+ stereocilia-like structures. These cells were able to rapidly uptake the styryl dye FM4-64, indicating functional mechanotransduction channels. We conclude that mammalian otic induction requires FGF signaling, which may be mediated by the ERK pathway. Future studies will include inhibition of parallel FGF-driven pathways in order to favor otic induction via ERK.
Background:
Traumatic brain injury (TBI), of which mild TBI (mTBI) accounts for more than 80%, significantly increases the risk of developing alcohol use disorders (AUDs) over a patient’s lifetime. Experimental mTBI has been shown to impair ethanol-induced behavioral sensitization and increase chronic and binge ethanol consumption/preference in the delayed post-TBI period. With its known relationship to ethanol consumption and physiological effects of ethanol, the endocannabinoid system has become of interest in AUDs secondary to TBI. Specifically, ethanol vapor and self-administration decreased striatal expression of cannabinoid receptor (CB) 1, and altered ethanol preference has been observed following CB1 antagonism in rodents. In this study, we investigated time- and region-dependent changes in CB1 and CB2 protein levels in the anterior striatum (ASTR) and nucleus accumbens (NAC) following mTBI.

Methods:
Anesthetized male C57BL/6 mice (8-10 wks) were given a mild, midline impact over the intact skull or sham surgery. At 8 and 25 d post-injury, brains were harvested and frozen. For Western blot analysis, protein lysates were made from 1.5 mm diameter tissue punches of striatal subregions taken from 2 mm thick coronal slices.

Results:
At 8 d post-injury, CB1 trended toward a decrease in ASTR, which became significantly reduced at 25 d post-injury. The NAC showed an initial increase in CB1 at 8 d post-injury, but was decreased at 25 d. CB2 protein levels did not differ at either time point.

Conclusion:
The observed time- and region-dependent alterations in CB1 expression in the ASTR and NAC may influence the development of AUDs in the post-TBI period.

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ALLOSTERIC MODULATION OF δ AND μ OPIOID RECEPTORS OCCURS BY A COMMON MODE OF ACTION
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Abstract. We have recently characterized positive allosteric modulators (PAMs) for the μ and δ opioid receptors (MOPr and DOPr, respectively). PAMs bind to a site on the receptor that is spatially distinct from the orthosteric site, which is defined as the binding site for the endogenous ligand. The binding of a PAM at the allosteric site can enhance the binding affinity and/or signaling output of endogenous opioid peptides as well as traditional opioid drugs occupying the orthosteric site. The therapeutic advantage of PAMs acting at MOPr and DOPr is their ability to provide pain relief by selectively enhancing the effects of endogenous opioid neurotransmitters at distinct locations involved in pain regulation, resulting in a reduced side effect profile. These PAMs may also be used as adjuncts to current opioid drugs. Additionally, PAMs acting at DOPr may have therapeutic potential as antidepressants. The mode of action of the MOPr PAM BMS-986122 was previously shown to occur by disruption of Na⁺ ion binding to the receptor, increasing the probability that the receptor will transition to active conformational states. We now show that BMS-986187, a structurally distinct PAM for DOPr, acts by a similar mode of action. The effect of BMS-986187 at DOPr was determined by comparing the modulatory effect of both BMS-986187 and Na⁺ ions on the binding affinities of a number of orthosteric ligands by radioligand competition binding using membranes from CHO cells expressing human DOPr. Functional assays measuring GTPγS binding as a readout for the influence of BMS-986187 on receptor activation were also performed. The activity and affinity of the tested ligands for DOPr were increased by BMS-986187 but the extent of these effects depended on the identity of the orthosteric ligand, a phenomenon known as probe dependence. For example, L-methadone showed a 180-fold increase in its binding affinity while the affinity of BW373U86 increased only 2-fold. Additionally, BMS-986187 was able to confer high affinity binding to DOPr for ligands that would normally bind with poor affinity, such as the endogenous κ opioid receptor agonist Dynorphin A (1-17). Comparing the sensitivity to Na⁺ and BMS-986187 for a number of ligands showed a positive correlation (r²=0.79) between affinity shifts due to the negative effect of Na⁺ on agonist binding and the positive effect of BMS-986187. These results indicate a conserved mode of allosterity at MOPr and DOPr, a finding that will prove useful in the development of new allosteric modulators for opioid receptors. Supported by R01 DA033397 and the Chemistry-Biology Interface Training Program.
Every year approximately 1.7 million individuals suffer the consequences of traumatic brain injury (TBI), which is a leading cause of death and disability in the United States. Due to a lack of effective clinical treatment, many of the functional and cognitive deficits sustained from TBI are left untreated. Currently, Phenelzine (PZ) is used clinically for the treatment of anxiety and atypical depression. Furthermore, PZ has been shown to express neuroprotective effects in several models of ischemiareperfusion and contusion brain injury. Additionally, research from our lab has shown that rats raised in enriched environment (EE) housing perform better during behavioral tasks than rats raised in traditional or standard laboratory environments (SE). The purpose of the current study was to examine the effect PZ administered post-injury may have on recovery of function following a medial frontal cortex (MFC) contusion in rats reared in EE housing.

Twenty-seven, twenty-five day old male Long-Evans rat pups were reared in enriched environments (EE) for ninety days. Twenty-seven adult male rats were purchased from the same vendor and placed into SE upon arrival. After fourteen days in SE, eighteen SE-housed rats received MFC contusion injuries. After Ninety days in the EE, MFC contusion injuries were administered to eighteen EE-housed rats. After each injury, at ten minutes post-injury, half of the animals received a subcutaneous (10mg/kg) injection of phenelzine, and the other half received an injection of saline solution. Behavioral analysis was conducted one week post-injury and included the open field task (OFT), Barnes maze (BM), Morris water maze (MWM), rotor-rod (RR), elevated-plus maze (EPM), and forced-swim task (FST). Upon completion of behavioral testing, the animals were euthanized and perfused, and their brains extracted. Tissue from the left hemisphere was embedded in paraffin, sectioned, and underwent hematoxylin and eosin staining. The right hemispheres were reserved for thick-sectioning analysis. Stereological analysis was performed to quantify total cortical volume as well as number of surviving cells in the hippocampus.

The data show significant differences between the intact and injured groups, for both EE-reared and SE-housed animals. In general there are no significant differences between TBI-treated and TBI-untreated animals that were raised in the EE. There are, however, significant differences between all intact, TBI-treated, and TBI-untreated animals that were housed in SE as adults.

Further exploration is needed to measure the influence that other factors, such as dosage, timing of administration, and housing environment may have on the efficacy of phenelzine as a treatment of TBI.
EFFECT OF SODIUM BENZOATE ON A RAT MODEL OF PARKINSON’S DISEASE

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Parkinson’s disease (PD) is the second most common neurodegenerative disorder after Alzheimer’s. L-DOPA, which is its main treatment, has side effects such as dyskinesia that become worse over time, and in some cases become more intolerable than the disease itself. A recent study (J Neuroimmune Pharmacol. 2014; 9:569-81) has shown that sodium benzoate (NaB), a metabolite of cinnamon, plays a neuroprotective role in a mouse model of PD. In the present study I examined the effect of NaB treatment on the 6-hydroxydopamine (6-OHDA) rat model of PD. The results show that rats who had NaB (150-300 mg/kg/day) in their water continuously beginning one week before the 6-OHDA lesion developed significantly less L-DOPA-induced dyskinesia (LID) as measured by the Abnormal Involuntary Movements (AIMs) scale after an injection of L-DOPA (25 mg/kg) and benserazide (15 mg/kg, i.p.) than rats who had regular water. The progression of LID between the first and second injections of L-DOPA was significantly lower in the group receiving NaB during the period between the injections than in the group that received regular water. To assess limb functionality and Parkinsonism, I measured the performance of both groups on the drag test. The NaB-treated group performed significantly better, indicating a possible neuroprotective or therapeutic effect of NaB. Finally, while previous findings were ambiguous about this subject, I show that LID due to high doses of L-DOPA correlates very well with the number of amphetamine (5 mg/kg, i.p.)-induced rotations in untreated 6-OHDA rats, but not in NaB rats. That NaB had an effect on the 6-OHDA rat model of PD, in addition to the MPTP mouse model, provides significant evidence that its mechanism of action possibly affects the universal underlying causes of PD, and not just model-specific mechanisms that give different models their parkinsonian-like phenotype.
FUNCTIONAL CONSEQUENCES OF AMINO ACID VARIATION IN PAIN-PATHWAY KV1.1 α- AND β-SUBUNITS IN A WILD RODENT (ONYCHOMYS) EXHIBITING REDUCED PAIN SENSITIVITY

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Pain is the most common reason American’s use the health care system and is the leading cause of long-term disability. By alerting the body to actual or potential tissue damage, pain acts as an adaptive mechanism. Unfortunately, pain can become maladaptive and interfere with daily tasks and quality of life. Our current understanding of pain dysfunction is insufficient for proper management and relief from pain. In the peripheral nervous system, pain signals are detected by specialized receptors and are carried by action potentials (AP) via their neurons to the spinal cord and brain. While sodium ion (Na+) channel opening initiates AP firing, opening of potassium ion (K+) channels acts as the ‘brake’ to prevent or terminate pain signals. Pathologic pain states are associated with hyperexcitability, or excessive firing, of sensory neurons leading to pain sensation. Rodent models support the fact that hyperexcitability in pain-like states is associated with altered expression and function of K+ channels. To better understand the role of K+ channels in pain signaling I am using a novel model system, the grasshopper mouse (Onychomys). Grasshopper mice prey on the bark scorpion (Centruroides), a species that produces painful and deadly toxins to ward off hungry predators. Their toxins specifically target Na+ and K+ channels, inducing burning pain and hypersensitivity to touch in humans. Grasshopper mice, on the other hand, display minimal pain-like behaviors in response to a scorpion sting and their sensory neurons exhibit decreased excitability compared to sensitive species (house mice, Mus musculus). This model provides an unprecedented opportunity to study the structure, function and expression of ion channels within the sensory pathway of a hypoexcitable system. RNA-Seq data, confirmed with Sanger sequencing, revealed amino acid substitutions in the pore-forming α- and auxiliary β-subunits of the voltage-gated K+ channel Kv1.1 in grasshopper mice compared to house mice. Two amino acid substitutions are located in the amino-terminus of the channel, responsible for tetramerization and association with the β-subunit. Five amino acid substitutions are present in the β-subunit in regions critical for ball-and-chain inactivation of K+ current. The Kv1.1 channel α- and β-subunits were cloned into the mammalian expression vector pcDNA3.1(+), and expressed in Chinese Hamster Ovary (CHO) cells. Whole-cell patch clamp techniques are being used to determine the time- and voltage-dependent properties of α-subunits alone or co-expressed α- and β-subunits, in order to determine how structural changes influence function. I predict that structural modifications to grasshopper mice Kv1.1 channels cause decreased neuronal excitability and, thus, modified pain signaling. Understanding how changes in K+ channel structure lead to changes in function will contribute to our understanding of pain state development.
Characterization of doxycycline inducible glutamatergic neurons

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The human brain develops through an elaborate succession of cellular events utilizing neurons and glia to construct neural circuitry that are tuned by the formation of plastic synapses. Subtle changes during neuronal development can disrupt this complex neuronal circuitry leading to neuropsychiatric disorders. With the advent of human induced pluripotent stem cell (iPSC) technology for converting adult cells into a pluripotent state comparable to that of embryonic stem cells (ESCs), we can begin to approach the understanding of complex neuronal development and neurological disorders in human cells. In the recent years, several “directed differentiation” protocols have been utilized to generate excitatory neurons. These protocols use growth factors and small molecules (SMAD inhibitors) that modulate developmental signaling pathways. Such protocols generally yield a heterogeneous population of cell types and require long-term culture to reach maturation. Recently it has been demonstrated that viral transduction of neurogenin2, a neurogenic bHLH transcription factor, in hiPSCs, combined with puromycin selection yielded populations of induced neurons (iNs) homogeneously expressing glutamatergic markers. In these studies, we adapted the induced neuron (iN) model using stable hESC clones under doxycycline control to understand the transcriptional and translational alterations that are involved in transition from stem cell to neuron. Over the time course of 11 days of dox treatment using RNA-Seq, we identified several key neuronal genes that were upregulated (MAP2, DCX, SYN, PSD95, VGLUT1, VGLUT2, CHGA, and CHGB) and pluripotency genes that were downregulated (NANOG, OCT3/4). The RNA-Seq data was confirmed using qPCR and the protein expression was correlated using western blotting. The morphological changes over the time course were also confirmed using immunostaining experiments for neuronal marker proteins. We have also generated hES cell lines that inducibly express neurogenin2 as well as GCamp6F which allow real time monitoring of spontaneous calcium activity in these neurons. Approximately 60% cells are able to spontaneously fire after 6 days of differentiation. Further investigation of spontaneous calcium activity and its impact of maturation of neurons and synapses are underway in our lab. We believe that these dox-inducible stable iN hESC clones will serve as a tractable model to understand the cellular events and the genetic changes that ultimately lead to formation of functional human neuronal circuitry.
Types of Music Differentially Impact Skin Conductance Response to Violent Stimuli

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Exposure to violent stimuli may cause desensitization and apathy towards real-life violence. While exposure to violent video games appears to lead to these issues, current research is varied regarding responses to music with negative messages. In order to determine if exposure to negative music induces desensitization to actual violence, skin conductance response (SCR) to a video of real-life violence was measured after exposure to either a positive song, a negative song, or no song, and compared to participants that did not observe the video. It was hypothesized that the group exposed to the negative song would respond less to the video of real-life violence compared to groups exposed to positive music or no music. Participants were recruited from a small liberal arts college and the surrounding community, with ages from 18-59, and included eighteen females and eight males. Pulse and SCR were used to measure heart rate and emotional response to the music, violent video, or control conditions. Pulse significantly increased after the video or control condition compared to baseline in all groups, but no group differences were observed. SCR for the negative song/video group and the no song/video group significantly increased during the song and video trials compared to baseline. SCR also significantly increased during the video trial compared to the song trial in no song/video group. As hypothesized, the negative song/video group exhibited desensitization to the video. Interestingly, those exposed to the positive song similarly did not display an elevated emotional response to the violent video, which may be indicative of a protective effect.

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DIAPEDESIS OF PAMAM DENDRIMERS ACROSS BLOOD BRAIN BARRIER WHEN ADMINISTERED THROUGH INTRACAROTID ARTERY IN C57BL/6J MOUSE MODEL


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Dendrimers are well defined 3-dimensional highly branched polymeric nanoparticles that are being widely used in biomedical application. The polyamidoamine (PAMAM) dendrimers can carry a large cargo that can be targeted to cells both in vitro and in vivo. One of the major issues of drug delivery into the central nervous system (CNS) is the presence of blood brain barrier (BBB), which is a complex multicellular structure that is selectively permeable to the molecules. It separates the CNS from the circulatory system. BBB consists of tight junctions made of mono layered endothelial cells that prevents leakage of plasma into the CNS. Molecules or drugs that are less than 1 nm in size are capable of passing through the BBB. Lipids and the lipid-based carrier molecules of larger size can cross the BBB by diffusing through the tight junctions but the rate of drug delivery is highly reduced. Hence it is difficult for most of the potential drugs or the biomolecules to enter a specific site in the brain. One of the widely used methods for drug administration into the CNS is by intracranial (IC) injection. This method has potential disadvantages such as localized administration of drug that does not reach a wide range of neural tissue that is usually needed to treat diseases like Alzheimer’s or Huntington’s disease in an effective manner. Drug delivery by IC injection requires invasive surgery, which has an increased risk and is more difficult technically to perform and often limits the number of administration of the drug, due to the safety concerns and discomfort associated with the multiple applications when this delivery protocol is used. With any potential treatment, route of administration and surface chemistry of the drug/biomolecule plays a vital role in the efficacy of the treatment as well as the clinical utility. Hence, to overcome the BBB and the complexity involved in intracranial injection, polymeric nanoparticles are being used to deliver drug systemically through blood vessels that can reach the central nervous system. New techniques that conjugate drug/biomolecules to the PAMAM dendrimers, which readily cross the BBB when administered via the carotid artery offers a viable alternative delivery mechanism. Our results show that in vivo administration of anionic PAMAM dendrimers of either 1-nm or 4-nm in diameter can cross the BBB when administered through the carotid artery and these, in turn, target the region of interest (such as the neostriatum) in C57BL/6J mouse model. This proof of principle suggests that drugs/biomolecules may provide an effective means of delivering therapeutics into the mouse models of, and, potentially, patients suffering from, Huntington’s disease.
Spinal cord injury (SCI) is characterized by the abrupt disruption of normal neuronal architecture, subsequently followed by cascading secondary tissue necrosis that further exacerbates the pathology. Current treatment strategies have been largely ineffective, but several new ideas have emerged, including cellular therapies, which show considerable promise. The idea for this project was to incorporate the role of stromal derived factor-1 (SDF-1), which provides a chemotactic gradient necessary for the normal development of many neuronal structures, as a means to guide migrating neuronal stem cells (NSCs) and/or growth cones towards their intended targets. Despite the strong migratory response induced by SDF-1 signaling, and the existence of its receptors, CXCR4 and CXCR7, on NSCs and on many growth cones, the possibility of applying this chemotactic molecule to induce regeneration has not been well characterized. This project utilized genetic engineering to force the overexpression of SDF-1 in mesenchymal stem cells (MSCs), which were used for subsequent transplantation into the epicenter of an SCI, with the goal of enhancing axonal ingrowth into the damaged area and to measure its behavioral consequences. The project included three, inter-related studies. The first study assessed whether or not axons surrounding the post-SCI environment contained CXCR4 receptors, and our findings indicated that axons in the dorsal column surrounding the lesion contained the CXCR4 receptor for at least 10 days post-SCI. The second study addressed the concerns of whether or not MSCs that are engineered to overexpress SDF-1 (SDF-1-MSCs) can enhance chemotaxic migration of NSCs in vitro beyond that of MSCs alone. By using viral construction and a transwell migration assays, we confirmed that SDF-1-MSCs can enhance migration of cultured NSCs. The third study addressed whether transplantation of SDF-1-MSCs into a contusive rat model of SCI can enhance the growth response of axons, and what influence this transplantation might have on behavioral recovery. Three transplantation groups were utilized, an SDF-1-MSC group, a MSC group, and a group receiving vehicle (Hanks Buffered Saline Solution). Results from this third study indicated that treatments with SDF-1-MSCs significantly improved behavioral outcomes on the Basso, Beattie, and Bresnahan (BBB) scale for locomotor recovery at 7 weeks post-SCI. Further, increases in immunological labeling of an axonal regenerative associated gene, GAP-43, were found in SDF-1-MSC treated rats. Based on these encouraging results, especially the findings of enhanced effects of SDF-1-MSCs on migration of cultured NSCs, we have initiated a study evaluating the co-transplantation of SDF-1-MSCs and NSCs, in which we have added three new groups: an NSC transplantation group, MSC-NSC co-transplantation group, and SDF-1-MSC-NSC co-transplantation group. Behavioral results on the BBB from this ongoing co-transplantation study have revealed that SDF-1-MSC-NSC transplantations improve behavioral outcomes greater than those observed in any of the other transplantation groups. Collectively, these findings suggest that SDF-1 secretion by MSCs, either on their own or in combination with NSCs, can significantly enhance the regenerative efficacy and reduce functional deficits in rats with SCI, and may provide a new strategy that could lead to more effective stem cell therapies for SCI.
ENGAGING IN PRAYER SIGNIFICANTLY DECREASES SELF-REPORTED ANXIETY AMONG CHRISTIAN UNDERGRADUATE STUDENTS

Introduction: Anxiety is a challenge that impacts nearly all human beings. However, as several research studies have indicated, there is a unique difference among those who cope with anxiety using prayer. Specifically, prayer and religious belief have demonstrated a negative correlation with anxiety. In an effort to further examine this relationship, a study consisting of a prayer session among individuals who associated themselves with prayer and religion was conducted.

Methods: At baseline, participants were prompted to rate their prayer frequency. From this data, participants were divided into two groups: high prayer frequency (daily) and low prayer frequency (ranging from once a week to once a month). To assess physiological and psychological changes related to stress, heart rate, skin conductance response (SCR), and self-reported anxiety were measured at baseline. After a prayer session lasting three minutes, heart rate, SCR, and self-reported anxiety were measured, and the participants were prompted to report if the experience impacted them in any specific way.

Results: A 2x2 multivariate analysis of variance indicated a significant interaction between trial and prayer frequency on reported anxiety ($p=0.018$). Post-hoc tests revealed that reported anxiety was significantly reduced in high and low prayer frequency groups after praying. Additionally, there was a significant effect of trial on SCR ($p=0.015$) indicative of an increased level of skin conductivity in participants in both high and low prayer frequency groups.

Conclusions: According to the results, it may be concluded that engaging in prayer is linked to a reduction in self-reported anxiety. However, participants, regardless of prayer frequency, tended to have higher physiological indications of stress after engaging in prayer according to their SCR. This may be attributed to feelings of elation rather than stress, or be related to pressure associated with participating in an experiment, being in a room alone, temperature, or other unknown variables.

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Recent research suggests that stress can lead to epigenetic changes that can be transmitted from parents to children and grandchildren. However, less is known about the transgenerational effects of gestational drug exposure. The current study was designed to test transgenerational effects of gestational anti-depressant exposure using a rodent model of maternal depression based on giving high levels of the stress hormone corticosterone for 21 days before pregnancy. “Depressed” (corticosterone-treated) or healthy female rats (F0 generation) received sertraline (a selective serotonin reuptake inhibitor; SSRI; 20mg/kg) or vehicle via oral gavage ~5 days prior to mating and continued the treatment until the end of gestation. The resulting F1 generation females were mated with new male rats in adulthood to produce the F2 generation that was investigated in this study. Male and female F2 rats were tested in the Forced Swim Test for depressive-like behavior and the Open Field Test for anxiety-like behavior. Preliminary results suggest a transgenerational effect of preconceptional stress hormone exposure on the F2 litter weights but no effect on the adult behavioral outcome and no effect of the gestational anti-depressant exposure.
To understand the basis of nervous system development, we must learn how multipotent progenitors generate diverse neuronal and glial lineages and how differentiation of progenitor cells into neuronal and glial cell fates is regulated. We addressed this issue in the zebrafish enteric nervous system (ENS), a complex neuronal and glial network that regulates essential gut functions. Little is currently known about how ENS progenitor subpopulations generate enteric neuronal and glial diversity. We identified spatially and temporally dependent progenitor subpopulations based on coexpression of three genes essential for normal ENS development: \textit{phox2bb}, \textit{sox10}, and \textit{ret}. Our data suggest that combinatorial expression of these genes delineates three major ENS progenitor subpopulations that reflect temporal progression of progenitor maturation during migration. We also found that differentiating neurons maintain \textit{phox2bb} and \textit{ret} expression, whereas they lose \textit{sox10} expression. Our data show that zebrafish enteric progenitors constitute a heterogeneous population at both early and late stages of ENS development and suggest that marker gene expression is indicative of a progenitor’s fate. Determining how other important marker genes colocalize with these three major subpopulations will reveal progenitor subpopulations that are important for specifying individual neuronal or glial subtypes. In addition, we use a set of zebrafish mutants with major defects in ENS development to identify novel genes and signaling pathways that are active in different ENS cell populations and during different developmental time points to regulate ENS development, which might also serve to uncover new genes involved in ENS diseases.
Behavioral sensitization to a low dose of cocaine and cross-sensitization to mephedrone in male Sprague-Dawley rats.

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Recreational use of synthetic cathinones (aka “bath salts”) is a significant public health concern. Previous and concomitant use with other psychostimulant drugs, such as cocaine, is commonly associated with illicit “bath salt” use. As such, previous exposure to other psychostimulants may enhance an individual’s sensitivity to the addictive properties of synthetic cathinones. Preclinical models are essential tools in psychopharmacology research on addiction. To date, very few preclinical studies have evaluated the effects of previous drug exposure on the abuse liability of synthetic cathinones. The current study implemented a behavioral sensitization paradigm to determine the effects of prior cocaine exposure on sensitivity to the locomotor stimulant effects of a common “bath salt” constituent, 4-methylmethcathinone (4-MMC). Seventy-two adult male Sprague-Dawley rats were administered subcutaneous injections of 5 mg/kg cocaine or saline once per day for five consecutive days. Locomotor activity was monitored on day 1 and day 5 one hour immediately before and one hour immediately after injections. After a ten-day washout period, rats were administered either saline or 4-MMC (1, 5, 10 mg/kg, S.C.) and locomotor activity was monitored in a similar manner to days 1 and 5 to assess the expression of cross-sensitization to 4-MMC. Cocaine produced increases in horizontal and vertical activity that were augmented with repeated exposure, indicative of behavioral sensitization. 4-MMC produced a dose-dependent increase in horizontal activity, with slightly higher activity in the cocaine-pretreated rats compared to the saline-pretreated rats. Measures of vertical activity showed a biphasic response to 4-MMC, with the greatest evidence for the expression of cross-sensitization between cocaine and 5 mg/kg 4-MMC. These findings suggest the possibility of increased abuse liability of 4-MMC in individuals with a history of prior cocaine use. To further evaluate this hypothesis, additional investigations may be warranted, including more direct behavioral indices of abuse liability and corresponding neurochemical measures.
Effects of Female Oviposition Choice in Nicotine Exposure on Neural Development and Behavior of the Fruit Fly (*Drosophila melanogaster*)

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Nicotine is a potent cholinergic alkaloid found in the nightshade family of plants, with tobacco being a primary source for this chemical worldwide. Nicotine is a chemical that functions as an anti-herbivory agent for the tobacco plant. However, recreational consumption of nicotine is widespread in human populations due to low level exposures associated with activation of brain regions associated with feelings of pleasure and reward in the dopaminergic pathways of the brain. The excitation of these pathways leads to potentially addictive consumption of nicotine. Pharmacologically, nicotine is a mimic of the the neurotransmitter acetylcholine. Current understanding of nicotine’s addictive properties have uncovered primarily through the use of mammalian models. However, limited assessment of the potential this chemical to affect growth, development and behavior in invertebrates has been undertaken at pharmacologically active, non-lethal dosages. Most invertebrate work has examined higher dosage exposures associated with insecticidal effects.

In this study, we show how we have modified an experimental design by Dodd to allow for environmental oviposition choices by female fruitflies, and we examine how chronic, low-level nicotine exposure choices in the fruit fly affects neurologically relevant aspects of development, morphology and behavior in comparison to control populations. These data are examined within the paradigm of examining multi-generational effects of a female’s oviposition choice and may be a suitable mechanism to identify epigenetic effects.
Origins of tetrodotoxin and molecular evolution in the voltage-gated sodium channels of poisonous newts (*Taricha granulosa*).

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Tetrodotoxin (TTX) is a potent neurotoxin that inhibits electrical signaling in excitable cells through selective block of voltage-gated sodium channels (VGSCs). Many diverse animals, including species of worms, crabs, octopuses, puffer fishes, frogs and newts, possess TTX as a defensive compound. However, rough-skinned newts (*Taricha granulosa*) possess the highest concentrations of TTX in any animal due to a coevolutionary interaction with TTX-resistant predatory garter snakes. Despite our extensive knowledge of the ecological consequences surrounding TTX toxicity in this system, the evolutionary origins of TTX, as well as the molecular basis for TTX resistance in the VGSCs of rough-skinned newts, have not been examined. Furthermore, the physiological properties of highly TTX-resistant VGSCs are largely unknown. In all other animals examined, TTX is produced by symbiotic bacteria inhabiting the skin and internal organs of host animals. We are characterizing the microbiome in *T. granulosa* using next generation sequencing and ecologically-guided cultivation to identify potential TTX-producing symbionts. To examine the evolution of TTX resistance in this lineage, we have also sequenced the VGSC genes in newts. We find numerous mutations in the highly conserved pore-loop regions, the sites where TTX binds the channel, in all six newt VGSCs. Overall, our research suggests that newts may serve as an excellent model system for understanding the roles of animal-microbial symbiosis in shaping adaptive evolution in the nervous system.
Immune Response Following Injury in the Adult Zebrafish Brain  
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The inherent plasticity and regenerative capacity of the zebrafish serve as a useful model for examining cellular interactions involved in the immune response following injury. Microglia are the resident immune cells of the central nervous system with dynamic processes that are able to respond to damage by migrating to the site of injury and phagocytizing pathogens and neuronal debris. We compared the microglial response following different forms of damage to the olfactory bulb in the whole fish versus the isolated brain in culture. Our previous findings suggested that direct injury to the olfactory bulb results in an acute immune response by resident microglia in the wounded bulb, while further proliferation may be due to migration of microglia from other regions of the brain or peripheral leukocytes entering through the olfactory nerves. We performed peripheral deafferentation and direct injury to the olfactory bulb in the whole fish and compared it to the isolated brain completely removed of all afferent input and peripheral influence. The olfactory bulbs of adult zebrafish were damaged by either cauterizing the olfactory organ or directly injuring the bulb with a stab wound. Complete removal of afferent input and peripheral influence was performed by isolating and culturing the brain for 4h in oxygenated artificial fish cerebrospinal fluid. Immunohistochemistry was performed following standard diaminobenzidine protocols using mouse monoclonal antibody 4C4 to label microglia.

Comparisons of the whole-fish treatment groups to untreated controls revealed that there was a significant increase in activated microglia in the damaged bulb following peripheral deafferentation (p<0.01). Following direct bulb injury, there was a significant increase in activated microglia in both the ipsilateral and contralateral bulbs (p< 0.01). In the isolated brains, there were significantly more activated microglia in the olfactory bulbs after 4h in culture than in isolated brains immediately after dissection (p=0.02). When the isolated brain in culture received a direct injury, there was a significant increase in activated microglia compared to the control (p<0.01). Thus, peripheral deafferentation and direct injury to the olfactory bulb results in different microglial response profiles in whole fish. In the isolated brain, there is a significant microglial response following 4h in culture, suggesting that microglia can respond to signals without afferent input or peripheral influence. Further work is required to explore the temporal differences in the degree of response in whole fish and the isolated brain following different forms of injury.
PREPARTUM SEROTONIN–SPECIFIC LESIONS TO THE DORSAL RAPHE ALTER VARIOUS ASPECTS OF MATERNAL BEHAVIOR AND REDUCE SEROTONIN FIBER DENSITY IN A SITE–SPECIFIC MANNER

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The postpartum period is characterized by increased maternal responsiveness, decreased anxiety, and increased aggression. Pharmacological manipulation of the serotoninergic system during the postpartum period alters these behaviors. In addition, our lab has recently found that lesioning serotonergic neurons in the dorsal raphe (DR), the main forebrain projecting serotonergic nucleus, after parturition decreases maternal aggression and pup-licking in laboratory rats. This evidence demonstrates serotonin’s importance during the postpartum period, but no studies have evaluated the function of serotonin during pregnancy, a highly sensitive period where hormone and peptide secretion induces changes in neurochemistry to initiate maternal responsiveness. Given serotonin’s role in facilitating hormone and neuropeptide release, I hypothesize that DR serotonin during pregnancy is particularly essential for the onset of postpartum changes in anxiety, maternal responsiveness, and maternal aggression. To test this, we destroyed serotonergic cells with a serotonin–specific neurotoxin (anti-SERT–saporin) infused into the DR during pregnancy. After parturition, we observed subjects’ maternal behaviors daily, including licking and nursing the pups, and maternal motivation was tested by conducting retrieval tests every other day. Additionally, anxiety-like behaviors were observed using two common anxiety paradigms, and maternal aggression was observed by placing a male intruder into the nest. DR lesions during pregnancy significantly reduced maternal aggression towards the intruder, similar to postpartum DR lesions. Surprisingly, the lesions had no effect on maternal behaviors during undisturbed observations; however, lesioned mothers did show increased maternal contact with pups immediately after disruption of the nest site during retrieval tests. The differences in behavioral findings between pre- and postpartum lesions suggest that serotonin’s influence on the display of maternal behaviors is diverse and dependent on reproductive state. Finally, serotonin fiber innervation was observed using immunofluorescence and confocal microscopy in several forebrain regions, including the central amygdala (CeA) and the medial preoptic area (MPOA). Preliminary analyses reveal a reduction in serotonin fiber density in the amygdala of lesioned subjects, but not the MPOA. These findings suggest that prepartum serotonin–specific lesions to the DR affect particular maternal behaviors by reducing serotonin output in a site–specific manner.
Proliferation of adult mammalian retinal neurons after application of an alpha7 nicotinic acetylcholine receptor agonist

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Irreversible vision loss is one of the top ten disabilities worldwide and is responsible for a reduced quality of life and a substantial burden on national healthcare systems. Glaucoma is a neurodegenerative disease in the retina that can lead to irreversible blindness due to loss of retinal ganglion cells (RGCs). Although the cause of glaucoma is unknown, the primary risk factor associated with the disease is an increase of intraocular pressure (IOP) and all current treatments are focused on reducing IOP. However, these treatments alone are insufficient to halt the progression of blindness associated with glaucoma, as RGCs continue to die even after IOP reduction. Regeneration of RGCs in adult mammalian retina could potentially reverse the effects of glaucoma. In this study, proliferation of adult Long Evans rat retinal neurons was examined after eye drop application of the alpha7 nicotinic acetylcholine receptor (α7 nAChR) agonist, PNU-282987.

Previous studies from this lab have used the α7 nAChR agonist, PNU-282987, for neuroprotection of RGCs in vivo using an induced glaucoma model in adult Long Evans rats. These studies reported that the α7 nAChR agonist was capable of preventing the loss of RGCs associated with induced glaucoma. Interestingly, these studies also indicated that some concentrations of PNU-282987 caused a significant increase in the total number of RGCs compared to internal controls. In this study, the proliferative effect of PNU-282987 on uninjured adult rat retinas was analyzed using 5-bromo-2'-deoxyuridine (BrdU). BrdU labels mitotically active cells during S phase. Previous studies have demonstrated the ability to regenerate rod, bipolar and amacrine cells to repair injured retinal tissue in mammals to a limited degree. However, here we demonstrate the regenerative effect of an α7 nAChR agonist without the necessity of prior damage or insult to the retina. Results are presented to support the hypothesis that new retinal cells originate from Muller glia. mRNA sequencing results are presented that indicate previously known retinal regeneration pathways, such as FGF2, may be involved in PNU-282987’s proliferative effect. These are the first studies to investigate generation of new retinal neurons in adult mammalian retinas as a result of an α7 nAChR agonist.

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Transplantation of human embryonic dopaminergic progenitors within the striata of PD patients has given the field encouraging results, but ethical concerns and tissue availability limit this approach. The use of mesenchymal stem cells (MSCs) and induced pluripotent stem cells as an alternative cell source for transplantation circumvents the ethical issues, and provides a readily available source of cells, as they are derived from adult tissue. To this end, we explored the use of MSCs as a cell source for DA neuronal induction prior to transplantation as a means to increase integration within the striatum. Our lab developed a novel adenovirus for the polycistronic expression of multiple genes (Ascl1, Lmx1a, and Nurr1) that are involved in DA neuron differentiation and used gfp to track transduction. MSCs were cultured with the adenovirus, which resulted in DA-like cells, which were verified based on protein, and gene expression, in addition to dopamine production. In the present study we transplanted these induced DA-neuronal-like cells into the striatum of rats who were given unilateral 6-hydroxydopamine (6-OHDA) lesions, a commonly used rat model of PD. The unilateral 6-OHDA lesion was assessed utilizing the cylinder test and by assessing the asymmetry of amphetamine-induced rotations. The induced DA-neuron-like cells, were transplanted into the dorsal striatum of rats at 8 weeks, following verification of the 6-OHDA lesion. After transplantation rats were divided into two groups, with one group sacrificed at 8 weeks and the second group at 24 weeks. There was a significant improvement in the cylinder test observed for both groups. A significant improvement in amphetamine-induced rotational asymmetry was observed in the 8-week group, whereas the 24-week group demonstrated a trend towards improved behavior in assessments of amphetamine-induced rotations. These results are suggestive of a potential clinical utility for this method.

Theme: C. Disorders of the Nervous System
Histamine is a biogenic amine that has been shown to function as a neurotransmitter in a number of invertebrate systems and is synthesized from its precursor, histidine, by the enzyme histidine decarboxylase (Hdc). In Drosophila, histamine is the neurotransmitter used by photoreceptors in the compound eye, and is also used by various mechanoreceptors as well as centrally located neurons. Mutations in the Hdc gene that have been previously isolated, such as HdcJK910, exhibit defects in histamine synthesis and display altered traits such as blindness, inability to groom, impaired temperature tolerance, and sleep. However, all of the Hdc mutants that have been obtained thus far have some residual histidine decarboxylase gene expression, leaving open the question what complete elimination of Hdc expression could cause in the fly. To determine the true null phenotype, an attempt was made to remove the Hdc gene via Minos transposon-excision mutagenesis of an existing Minos-type transposon that is located within the Hdc gene. Excision mutagenesis is typically achieved by crossing a fly carrying the Minos transposon with a fly carrying the Minos-specific transposase gene and activating transposition of Minos in their progeny. The Minos transposon that was used contains the gene for green fluorescent protein (GFP); therefore its presence or absence can be used to visually identify a potential excision event. Once a fly was identified as missing the Minos element (lacking GFP), a genetic line was established and the progeny then further examined using histamine immunostaining to determine the presence or absence of histamine in the specific line. Given the nature of transposon excision mutagenesis, expected results should include the following expected categories: (1) flies contain wild-type levels of histamine, indicating a precise Minos excision; (2) flies contain trace levels of histamine, indicating a disrupted transposon left at the excision site; (3) flies containing no histamine, indicating imprecise excision and disruption of the Hdc gene. The strains that have no detectable histamine were crossed with the HdcJK910 mutant strain and their progeny examined for histamine, with a negative result confirming a deletion of Hdc. Our results thus far include strains falling into each of the 3 expected categories, and a number of histamine negative strains have shown no histamine when crossed to HdcJK910. Results thus far indicate that several new Hdc deletion mutants have been isolated in Drosophila melanogaster, which will now be examined to identify precisely where in the gene the disruption has occurred.
Title: Persistent pro-depressant effects of adolescent nicotine exposure in male rats

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Abstract: Nicotine use among adolescents remains a significant public health issue. Adolescence is a critical period for neural development and nicotine exposure during this time may significantly alter the trajectory of behavioral and neural development. Animal studies have shown that chronic nicotine administration in adolescence leads to increased immobility time on the forced swim test in adulthood. We explored the persistent effects of adolescent nicotine exposure on other depressive-like behaviors, anxiety-like behaviors, and basal neural activity. Male Sprague-Dawley rats received sub-cutaneous injections of 0.1 mg/kg nicotine or saline twice daily during adolescence (postnatal days 28-45). In adulthood, rats were subjected to the forced swim test, the sucrose preference test, the elevated plus maze, or were sacrificed for brain harvesting. Brains were sliced to a thickness of 20 μm and stained with cytochrome oxidase to provide an indication of basal neuronal activation. Images of stained slices were inverted and analyzed using region of interest (ROI) analysis in ImageJ. Signal intensity for each ROI was adjusted via background subtraction. Relative to saline-treated animals, nicotine-treated rats showed increased immobility time on the forced swim test and a decreased preference for 1% sucrose solution in adulthood. However, nicotine-treated and saline-treated rats did not differ in time spent in the open arms of the elevated plus maze or in the number of entries made into the open arms. Nicotine-treated rats showed decreased basal neuronal activation in the prefrontal cortex, nucleus accumbens, and caudate/putamen compared with saline-treated rats. Increased basal neuronal activation was observed in the amygdala of nicotine-treated rats compared with saline-treated rats. Taken together, these findings suggest that nicotine exerts long-lasting increases in depressive-like behavior but not anxiety-like behavior. Repeated nicotine exposure during adolescence was also associated with changes in basal neural activation in brain areas associated with motivation, reward, and emotion. Future studies will address whether these effects are specific to adolescence or may also be observed following chronic nicotine exposure in adulthood.
VULNERABILITY OR RESISTANCE: CONTRIBUTION OF UCH-L1 IN MESOLIMBIC AND NIGROSTRIATAL DOPAMINERGIC NEURONS

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Nigrostriatal dopamine (NSDA) neurons degenerate in Parkinson disease (PD), a debilitating movement disorder affecting 1 million people in the United States. Certain other DA neuronal populations in the brain are resistant to neurodegeneration. The mesolimbic dopamine (MLDA) neurons, which comprise a key neuronal pathway in reward and addiction, are one such population that does not degenerate in PD. Previous studies have shown that MLDA neurons are less susceptible to DA loss in the acute MPTP model compared to NSDA neurons, which offers a convenient paradigm to study pathways involved in neuroprotection that recapitulates sparing of MLDA neurons. In patients with PD, inhibition of the ubiquitin proteasome system (UPS) contributes to neuron loss in NSDA neurons, which results in accumulation of toxic aggregates. Not surprisingly, key players in the UPS are mutated in familial forms of PD, such as ubiquitin carboxy-terminal hydrolase L1 (UCH-L1). UCH-L1 is a neuron-specific, highly abundant deubiquitinating enzyme tasked with maintaining pools of monomeric ubiquitin and ensures that adequate supply of ubiquitin is available so that targeted proteins may be tagged and degraded by the 26S proteasome. In post-mortem brains of PD patients, UCH-L1 is downregulated and oxidatively modified, providing a clue that loss of UCH-L1 could contribute to the destruction of NSDA neurons. UCH-L1 expression is also decreased in mouse substantia nigra (cell body region of NSDA neurons) after MPTP treatment; therefore, the decrease in UCH-L1 expression and function in the NSDA neurons is hypothesized to contribute to loss of DA and tyrosine hydroxylase expression with MPTP treatment. In the ventral segmental area (VTA, cell bodies of MLDA neurons), UCH-L1 is not decreased with MPTP treatment, suggesting that one of the ways MLDA neurons cope with dysfunction resulting from neurotoxicant treatment could be by maintaining UCH-L1. Further characterization of UCH-L1 expression and function in MLDA versus NSDA neurons is needed to test this hypothesis, including measurements of UCH-L1 expression and activity in the nucleus accumbens (NAc, axon terminals of MLDA neurons) versus the dorsal striatum (axon terminals of NSDA neurons). These experiments will yield insight into the ability of UCH-L1 to contribute to neuroprotection of MLDA neurons in the face of neurotoxicant exposure, which can be explored for future therapies to prevent or halt neurodegeneration in PD.
Mild traumatic brain injury (mTBI) affects more than 1 million Americans each year, accompanied by short-term and sometimes long-term changes in cognitive functioning. However, the etiology of these changes still requires some elucidation. Investigation of cerebral blood flow (CBF) and brain functional connectivity together, as biomarkers of brain metabolism and activity, may provide some insight into how brain function changes following mTBI. A single MRI sequence, PASL, can provide us a unique opportunity to simultaneously assess both CBF and intrinsic connectivity networks (ICNs) due to its sensitivity to both static and dynamic CBF. We performed PASL in 30 healthy individuals and 22 mTBI patients at the acute stage, generated individual subject CBF maps and extracted the default mode network (DMN) from the PASL time series using independent component analysis (ICA). A voxel-wise nonparametric t-test with family-wise error (FWE) correction showed increases in CBF in mTBI patients, which mainly overlap with the default mode network (DMN). The ICA showed reductions in connectivity within the DMN, similar to our previous findings using rsfMRI; however, this did not survive FWE correction. In conclusion, identification of similar networks from PASL as from rsfMRI suggests that ICNs can be identified from PASL data. Moreover, because the PASL signal is mainly derived from blood flow, PASL ICNs may be more representative of brain metabolism than blood oxygen level dependent (BOLD)-based ICNs, since BOLD is also affected by other physiological parameters, while also providing extra information from the PASL data at essentially no cost. The combination of decreased connectivity within the DMN and increased CBF in some regions of the DMN may represent an acute brain response to injury. We plan to further investigate this using a method more specific to oxygen consumption.
Methylmercury perturbed the antioxidant system in spinal cord motor neuron like cells.

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Abstract

Pathogenesis of environmental neurotoxicant methylmercury (MeHg)-induced toxicity includes the perturbation of intracellular \([Ca^{2+}]_i\) homeostasis and antioxidant system. Previous studies from our lab demonstrate that chronic postnatal exposure to MeHg at doses which did not produce overt neurotoxicity, markedly enhanced development of amyotrophic lateral sclerosis (ALS)-like phenotype in a susceptible mouse model. These ALS-brainstem motor neurons (MNs) demonstrated dysregulation of \([Ca^{2+}]_i\) homeostasis. Additionally, acute MeHg exposure altered \([Ca^{2+}]_i\) homeostasis in wildtype mouse MN primary cultures. As a means of studying the effects of MeHg further on MNs, we sought to characterize its effects in the neuroblastoma spinal cord motor neuron (NSC34), a MN-like cell line and spinal cord cell culture (SCC) to use as MN model systems. Chronic exposure to 0.5\(\mu\)M MeHg in NSC34 cells showed a significant reduction of cell viability when exposed for 72 h. This reduction of cell viability is apparently associated with the loss of antioxidant homeostasis during MeHg exposure. This includes an increase in mRNA expression of Gclc (glutamate cysteine ligase catalytic subunit) which is the rate-limiting enzyme for glutathione synthesis and Txnrd1 (thioredoxin reductase1), which is the reactive oxygen species detoxification enzyme. The \(Gclc\) and \(Txnrd1\) mRNA increase occurs in a time-dependent manner. On the other hand, acute MeHg exposure to a concentration ranging from 1, 2 and 5 \(\mu\)M MeHg for 24h showed concentration-dependent MeHg-toxicity. We further investigated the effect of dimethylfumarate (DMF, Tecfidera ®, a drug that activates the antioxidant pathway and used for multiple sclerosis treatment). Pretreatment or co-treatment of DMF in NSC34 cells or SSC inefficiently protected MN degeneration following 2\(\mu\)M MeHg exposure. This is partly due to the short half-life of DMF. NSC34 cells treated with 7, 21 and 42 \(\mu\)M DMF for 24 and 48 h greatly decreased the expression of antioxidant \(Nqo1\) and \(Txnrd1\) mRNA after 48 h of treatment.
Lateral hypothalamic neurotensin neurons engage the mesolimbic dopamine system to regulate water intake and locomotor activity

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The lateral hypothalamic area (LHA) acts in concert with dopamine (DA) neurons in the ventral tegmental area (VTA) to regulate the motivation to feed, drink and move. It remains unclear, however, how specific LHA neuronal populations modify ingestive and locomotor behaviors. We examined how LHA neurons expressing the neuropeptide neurotensin (Nts) engage the DA system to regulate behavior. LHA Nts neurons project to the VTA, where many DA neurons co-express neurotensin receptor 1 (NtsR1). Furthermore, these VTA NtsR1-DA neurons project to ventral striatal brain regions that regulate ingestive behavior and locomotor activity, such as the nucleus accumbens (NA). Selective activation of LHA Nts neurons using DREADD technology increases pCREB expression in the NA, confirming that LHA Nts neurons functionally modulate the mesolimbic DA system. Next we examined the physiological role of this circuit by activating LHA Nts neurons in normal mice (WT) and in mice that lack NtsR1 (NtsR1KO mice). Activation of LHA Nts neurons increased locomotor activity and oxygen consumption in WT and NtsR1KO mice, but these effects were blunted by the DA receptor 1 (DR1) antagonist, SCH23390. Acute activation of LHA Nts neurons did not alter chow intake in sated WT mice, but it significantly increased their water consumption. Intriguingly, drinking behavior was not blunted by inhibition of DR1, but was suppressed in NtsR1KO mice, suggesting a specific role for NtsR1 in ingestive behavior. Together, these data reveal that LHA Nts neurons engage the mesolimbic DA system to modify drinking and locomotor behaviors via distinct signaling mechanisms. Intact action via the LHA Nts neuronal circuit is thus crucial for coordinating physical activity and intake of natural rewards, such as water.
Genetic instability of the polyglutamine tract in a mouse model of spinal bulbar muscular atrophy
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Spinal bulbar muscular atrophy (SBMA) is a neurodegenerative disorder that exhibits myopathic and neuropathic characteristics, including muscular weakness and atrophy and lower motor neuron loss in the brainstem and spinal cord. The molecular basis of this disease is a polyglutamine (CAG) expansion in the androgen receptor (AR) gene, resulting in a misfolded and dysfunctional protein. The AR113Q mouse is a popular model used to study SBMA. This model contains 113 CAG repeats in the AR gene, and results in decreased motor function and survival of these animals.

Polyglutamine tracts are notoriously unstable, and can expand or contract in subsequent generations in both humans and animal models. This has been documented in several other polyglutamine diseases, including Huntington disease and several forms of spinocerebellar ataxia. The instability of the polyglutamine tract is thought to occur through a process known as meiotic or intergenerational instability, of which the mechanism remains largely unknown. Little research has been done on this genetic instability in the context of SBMA. We analyzed the instability of the polyglutamine tract through 3 generations of mice in the AR113Q colony at CMU and its effect on the SBMA phenotype of these animals. With maternal transmission, we found significant differences in CAG repeat tract length in each generation. The mean repeat change of the first generation was -0.35 (range -2 to +1), second generation was -4.246 (range -10 to +1), and the third generation was -0.81 (range -6 to +3). Surprisingly, we found no sex differences related to the tract length change in the progeny. We also found that the contracted CAG tract no longer produces a motor deficit in AR113Q mice, but mice still have a shortened lifespan. Finally, we saw a trend of increased protein levels of some AR-interacting coactivator proteins (steroid receptor coactivators 1-3) in our AR113Q-KI mice, though this change was only significant for steroid receptor coactivator 2. In conclusion, the CAG tract instability observed in the AR113Q knock-in mice has a diminishing effect on the SBMA phenotype, though some changes are still observed at the molecular level.
Investigation of the effect of lithium on oxidative stress and DA-induced toxicity produced by MPP+

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Parkinson disease (PD) is the second most common neurodegenerative disorder that is characterized by selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), which results in a host of motor disturbances. One of the possible causes of idiopathic PD is toxicity of dopamine (DA) to neurons. DA is a highly reactive neurotransmitter that has been shown to produce reactive oxygen species and oxidized metabolites, which could lead to DA-induced neurotoxicity. Due to the high concentration of DA physiologically present in dopaminergic cells, both intracellular and extracellular DA dynamics need to be maintained. It has been reported that elevated extracellular DA induces cytotoxic effects via oxidative stress. In addition, elevated intracellular DA has been shown to inhibit mitochondria complex I and other proteins to cause cellular dysfunction and cell death. Two important proteins regulating intracellular and extracellular DA levels are vesicular monoamine transporter 2 (VMAT2) and the dopamine transporter (DAT). VMAT2 transports DA from cytosol to synaptic vesicles for storage until it is ready to be released at synapses, while the DAT tightly regulates extracellular DA levels by causing its reuptake into presynaptic neurons. VMAT2 and DAT are lucrative targets for pharmacological enhancement to maintain intra and extracellular DA dynamics, which could slow or halt neurodegeneration in PD. Lithium is an FDA approved anti-mania drug used for the treatment of bipolar disorder that has been shown to be protective against oxidative stress effect by inhibition of glycogen synthase kinase 3 (GSK-3) signaling pathways. Various studies have suggested that GSK-3 inhibition is involved in resistance of oxidative stress but its effects on VMAT2 and DAT in maintaining DA concentration is unknown. 1-methyl-4-phenylpyridinium (MPP+) is a neurotoxicant that selectively enters dopaminergic cells via DAT and inhibits mitochondria complex I to cause cell death. Our previous study showed that the MPP+ increased the ability of dopaminergic cells (MN9D) to compensate for intracellular DA loss by increasing tyrosine hydroxylase (TH) following 24 hours exposure. 40Ser-phosphorylated TH (p40-TH) will be measured for markers of active form of TH. 3,4-dihydroxyphenylacetaldehyde (DOPAL), nitrotyrosine, and 4-hydroxynonenal (4-HNE) will be measured as a marker for marker of oxidative stress. The effects of lithium on VMAT2 and DAT levels will also be measured. The MN9D cell line will be used to investigate the effects of lithium and MPP+ on oxidative stress and DA-induced toxicity after MPP+ exposure.