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Poster Abstracts

The Effect of Ketamine on Socially Isolated Stressed Mice

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Social isolation in the mouse model can impair mental health in early adolescence.Single-housed (SH) animals show a higher susceptibility to depression and anxiety-like behaviors compared to group-housed (GH) animals. The effects of social deprivation are seen asneuroanatomical differences in the prefrontal cortex and hippocampus. Ketamine, an NMDA receptor antagonist, has been shown to alleviate depression symptoms, giveimmediate results and have longer-term benefits than many other treatments of depression. Ketamine has been shown to increase BDNF levels and synaptogenesis in the cortex. To further elucidate the mechanism of action of ketamine, the current study examined the impact ketamine treatment upon depressive-like behavior of SH and GH mice, based on the forced swim test. The behavioral analysis of the forced swim test before and 1 day after ketamine treatment (10 mg/kg, IP) revealed no change for the GH mice. In contrast, the forced swim test revealed a ketamine effect on the SH mice. The SH mice that were in the upper 50-percentile for depressive-like behavior (N=9), as compared to the GH group (N=6), showed significant decrease in immobility following ketamine treatment (p<0.05). Our hypothesis is that ketamine alleviates depression through alterations in trafficking of synaptic proteins into spines involving drebrin. We chose to study the role of drebrin, because it has been shown to modulate the activity-dependent trafficking of NMDA receptors in spines. We used electron microscopic immunocytochemistry to quantify levels of drebrin within spines of prefrontal cortex of SH mice with ketamine versus vehicle saline treatment. Preliminary EM data with a sample size of 4 per group shows no difference in spine density between the saline and ketamine groups but a decrease in the proportion of spines in layer 1 with drebrin immunoreactivity for the ketamine- treated group (p<0.05). Only 1 of the 4 mice analyzed in the ketamine group had a significant reduction in depressive-like behavior but additional animals with reductions in depressive-like behavior are planned in the near future. Further research will test whether ketamine plays a role in drebrin-mediated trafficking of synaptic proteins as the mechanism of action in changing prefrontal cortical circuitry underlying depression.

The role of corticostriatal interactions in complex movement.

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Moving across dynamic surfaces and obstacles requires integrating detected cues and movement selection. Cue detection is proposed to occur through interactions between cortical cholinergic and glutamatergic transients. Precisely, cortical cholinergic transients are thought to mediate cue detection in situations requiring an attentional shift from a cue monitoring state to cue-guided behavior. Failures in cue reporting termed misses can be detrimental in cue-guided behavior; for example, missing cues while walking can lead to life-threatening falls and hospitalization. Cue detection via cortical cholinergic signaling has been extensively studied; however, the subsequent recruitment of downstream regions, such as the striatum, is relatively unexplored. We propose that cortical glutamatergic projections inform the striatum of cue-related information to direct behavior. To tackle this hypothesis, we recorded from animals with opposing attentional styles and cortical cholinergic signaling capacities, sign-trackers (STs), intermediates (INs), and goal-trackers (GTs). STs rely on bottom-up/cue-driven attention, while GTs exert top-down/goal-driven attentional control. This difference is thought to be mediated by unresponsive versus elevated levels of cortical cholinergic signaling, respectively. We recorded via glutamate-sensitive biosensors dorsomedial striatal glutamatergic signaling in rats trained to perform a cue-triggered turning task. Rats utilize one cue (indicating reversal in the treadmill belt) to turn before the treadmill restarts and another cue to wait until the treadmill restarts in the same direction. Recordings were time-locked to task events with trials video-recorded for off-line behavioral analyses. Preliminary electrochemical data from these rats suggests that transient glutamatergic activity coincides with specific task events in a phenotypic-dependent manner-activity was elicited in STs in all trials. In contrast, in GTs, activity was elicited only in successful cue detection. This suggests that selective signaling in GTs may account for superior attentional regulation due to increased non-relevant noise filtering. Together, these results highlight phenotypic differences in cue-directed behavior and may elucidate the neural correlates of attentional control in complex movement.

Experience-dependent encoding of threat stimuli and defense by Lateral Septum Crfr2 neurons.

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Perception of controllability over stressful stimuli is critical for engaging adaptive behavioral coping strategies in response to future threats. However, how the brain processes threat stimuli under controllable or unavoidable conditions to guide defensive behavior remains elusive. Well-positioned to mediate complex aspects of threat encoding are lateral septum (LS) neurons expressing the stress-related neuropeptide receptor gene, Crfr2, which are activated by and influence responses to innate threats. Here, using in vivo fiber photometry, we show that the that LS Crfr2 activity patterns are associated with distinct threat-related variables depending on the animal's performance in a conditioned active avoidance task where mice perform avoidance behaviors in response to conditioned stimuli (CS) predicting punishment. We find that these differences in neural activity patterns are due to the animal's perception of controllability of the aversive shock outcome - responses to the CS develop over training but diminish after animals develop avoidance responses. Surprisingly, LS Crfr2 population activity also rapidly increases at the time of each avoidance response â€“ a neural response we find is required for the expression of these active defensive behaviors. Furthermore, using microendoscopic calcium imaging, we revealed that the population activity reflected heterogenous neuronal dynamics that can be clustered based on their contribution to distinct task-relevant variables (i.e. CS, avoidance, and punishment). We are currently testing whether distinct clusters represent behaviorally or physiologically-relevant learning rules, how each cluster is represented in individual animals dependent on their performance, and probing hypothalamic afferents that may serve as sources of threat-related information. Together, these findings elucidate a neural mechanism by which differences in threat perception and experience promote distinct defensive behaviors. Funding Acknowledgement: This work is supported by the NSF DGE1745303 (DLB) and NIH R01MH117421 (TEA).

The central nucleus of the amygdala is necessary for maintenance of fentanyl self-administration in rats

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Opioid use disorders (OUD) are initially driven by activation of reward pathways, but hypothesized to persist due to recruitment of aversive pathways in the brain that result in a negative affective state. One brain region implicated in these adaptations is the central nucleus of the amygdala (CeA), which has been shown to play a key role in multiple substance use disorder and anxiety models. Despite evidence of the role of the CeA in substance use disorders, there has been limited exploration of its role in OUD. To assess the role of the CeA in opioid self-administration we trained male and female Sprague-Dawley rats to self-administer the synthetic opioid fentanyl (0.5Î¼g/infusion) in a 2-hour FR1 self-administration paradigm. Rats reached a stable and relatively high level of opioid infusions after training. Using reversible inactivation with the GABA agonists baclofen and muscimol, we found that inactivation of the CeA significantly attenuated the number of fentanyl infusions and altered the pattern of consumption across the session. We additionally micro-infused the non-specific opioid receptor antagonist naltrexone into the CeA to assess the role of CeA opioid receptors in fentanyl intake. This resulted in increased fentanyl intake across the session, indicating that opioid receptors in the CeA contribute to fentanyl intake. These results are consistent with theories that the CeA plays a critical role in continued drug consumption in OUD, and further indicate that CeA opioid receptors play a key role in fentanyl reward.Supported by R01DA035943 and R01AA026306.

A novel approach to rule-learning in mice using operant touch screen boxes.

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In the past, higher order cognitive ability has been difficult to assess in rodent models. So far, cognitive abilities such as rule generation and complex decision making have not been evident in mice, rats, and rabbits. Rats have shown marginal evidence of being able to comprehend information and transfer information from one set to another through tasks such as attentional set shifting. Mice, however, have not shown any ability to perform this task. In this experiment, we are analyzing whether mice can learn a visual discrimination task, increasing in complexity, and then apply this remembered rule to a different image set. 28 wild-type male c57 mice performed the visual discrimination, conducted in Bussey-Saksida touchscreen operant boxes, on 2 different image sets, counterbalanced on which image set each group received first. Results have shown that these mice were able to learn and remember the rule from their first image set with low difficulty, however, were unable to transfer this to a new set of stimuli. The development of this task is still ongoing, with the stimuli and the presentation of which all subject to change. The work on this project was supported by the Institute of Brain and Behavioral Sciences (IBACS) to the Murine Behavioral Neurogenetics Facility (MBNF).

Assessment of seizure and sleep pathology in a genetically engineered mouse model of sporadic Alzheimer's disease

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Alzheimer's disease (AD) is the foremost cause of dementia and fifth leading cause of death worldwide. The financial toll of AD is expected to double every 20 years, placing severe burdens on worldwide health infrastructure. Now 115 years from the first descriptions of pathology in 1906, there are no disease modifying treatments. Recent evidence suggests intervention at the earliest stages of amyloid seeding may prevent accumulation and AD progression (Uhlmann, 2020). Non-convulsive epileptiform activity is prevalent, detectable before amyloidosis, and predicts cognitive decline in AD. Electroencephalography (EEG) is useful for detecting these events as well as sleep disturbances that exacerbate memory decline in AD. We assessed epileptiform activity and sleep patterns in a novel mouse strain modeling sporadic genetic risk factors of AD. The sporadic Late Onset AD (LOAD) mouse used in the present studies was genetically engineered using CRISPR/Cas9 and was homozygous for humanized sequences in ApoE4, homozygous for the LOAD risk variant TREM2 R47H, and heterozygous for the humanized APP sequence. Our hypothesis was that the LOAD mouse may demonstrate abnormal EEG signals in line with that reported for LOAD patients prior to significant pathology and cognitive decline. Telemetric EEG and continuous video recordings were conducted at an age prior to significant pathology or cognitive impairment (12 months). These mice were compared to age-matched C57BL6/J mice. Recordings were scored for epileptiform spiking, sleep staging, and power distribution during wake and sleep. While LOAD mice did not exhibit increased epipleptiform activity ($t(13)= 0.22$, $p=0.83$) they did exhibit EEG alterations relative to controls including increased inactive phase REM sleep ($t(13)=2.76$, $p=0.02$), decreased delta and increased alpha power during wake ($F(4, 65)=5.210$, $p<0.01$), as well as decreased delta power ($F(4,65)=3.90$, $p<0.01$) and a rightward shift in EEG power during REM. Interestingly, although the sleep or seizure phenotypes were modest at the current ages of the LOAD mice studied, the phenotypes detected in the LOAD sleep patterns may implicate an early phenotype preceding significant neuropathology and cognitive deficits in these mice, similar to that reported in patients. These findings warrant more comprehensive assessments in mouse models of LOAD which may better recapitulate early neurological anomalies as predictors of AD onset. Supported by NIA U54 AG054345-01.

Investigating neuroadaptations in the basolateral amygdala underlying sex differences in cue-induced cocaine craving

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Cocaine addiction is a devastating public health epidemic that continues to grow. Sex and ovarian hormones are known to influence relapse vulnerability but little is known regarding the cellular and synaptic mechanisms contributing to these sex differences. To investigate this we use a rodent model of cocaine craving and relapse called the incubation model in which cue-induced seeking progressively increases or â€œincubatesâ€• during the first month of withdrawal from extended-access cocaine self-administration. We have recently shown that increased activity in the basolateral amygdala (BLA) is associated with changes in incubated cue-induced cocaine seeking behavior following 2-3 weeks of withdrawal in male rats. However, less is known regarding the cellular and synaptic mechanisms driving changes in BLA activity and whether this is influenced by sex or hormonal fluctuations across the rat reproductive (estrous) cycle. To investigate this, we have conducted western blots to assess changes in glutamate receptor levels (GluA1, GluA2, mGlu5, mGlu1) in adult male and female Sprague-Dawley rats following 2 weeks of withdrawal from extended-access cocaine or saline self-administration. Ex vivo whole-cell patch clamp recordings from BLA pyramidal neurons were also conducted at similar time points. All data were analyzed across different stages of the rat estrous cycle. Our data indicate sex differences in glutamate receptor expression and BLA excitatory synaptic transmission during the first month of withdrawal from extended-access cocaine self-administration that are influenced by estrous cycle fluctuations and that may contribute to known changes in incubated craving across the estrous cycle. Together these studies will help identify mechanisms that could be driving sex differences in cue-induced relapse vulnerability.R00 DA038110 to J.A.L.

Stress-induced sex-specific plasticity of prefrontal parvalbumin neurons contributes to increased vulnerability of female mice to anxiety behaviors.

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Anxiety disorders often emerge after exposure to a stressful event, and women are twice as likely as men to develop such a stress-induced mood disorder. The mechanisms underlying this sex-specific vulnerability remain unclear. Our previous chemogenetics data indicate that increased activity of prefrontal parvalbumin (PV)-expressing neurons modulates anxiety-like behaviors in female mice. We are now aiming to test the idea that stress-induced sex-specific plasticity of these neurons determines the extent to which chronic stress leads to sex-specific emotional dysregulations. Exposure to a short period (4 weeks) of unpredictable chronic mild stress (UCMS) induces anxiety-like behaviors in female mice, while a longer exposure (8 weeks) is necessary to induce a similar anxious phenotype in males. Here, we use behavioral and ex vivo electrophysiology approaches to correlate stress-induced changes in prefrontal PV-neurons activity with emotional dysregulations after UCMS exposure. As predicted, females showed anxiety- and depressive-like behaviors after 4 weeks of UCMS, while males display anxiety only after an 8-week exposure. Our preliminary electrophysiological data show that UCMS history makes resting membrane potential of PV neurons less negative, emerging at 4 weeks in females and 8 weeks in males, and action potential (AP) width is reduced after 4 and 8 weeks of UCMS in females. Voltage-gated potassium channels, such as the Kv3.1 ones, regulate the fast-spiking properties of PV-neurons by shortening AP duration. We therefore suggest that UCMS-induced changes in Kv3.1 expression drive vulnerability to stress-induced anxiety behaviors in females. We used Kv3.1 knock-out (KO) female mice that have PV-neurons with increased AP width. As predicted, Kv3.1 KO mice display full resilience to UCMS-induced anxiety-like behaviors. Altogether, our data provide further evidence that UCMS-induced changes in prefrontal PV neurons firing properties contribute to sex-specific vulnerability to anxiety, highlighting a novel key mechanism underlying vulnerability to stress-induced emotional dysregulation. Funded by NIH grant R21MH119090.

Role of RGS7 in the regulation of Kappa Opioid Mediated Aversion.

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The kappa opioid receptor (KOR) system is a prominent neuromodulator system that controls negative affective states and represents a central player in the regulation of aversion. KOR belongs to the superfamily of G protein-coupled receptors (GPCRs) whose signaling efficiency is controlled by regulator of G protein signaling (RGS) proteins. Our understanding of the molecular diversity of RGS proteins that control KOR signaling is limited. In this study we used a genetic approach to target the R7 RGS family, a family highly enriched in the brain which regulates GÎ±i/o-coupled receptors to decipher its role in KOR-mediated behaviors. We used a global knockout of R7BP, a common binding partner that is required for the stability of R7 RGS proteins in conditioned place aversion (CPA) behavioral assays to assess the mechanism underlying KOR-dependent dysphoria. R7BP knockout mice showed an enhanced aversion to the KOR agonist U50, 488 compared to their littermate controls. Specific targeting of individual R7 RGS members revealed that Rgs7 is responsible for the KOR-mediated aversion. Together, this study identifies Rgs7 as a novel regulator of KOR signaling and pinpointing its role in regulating aversion.

Prenatal circadian rhythm disruption induces sex-specific substance use-related phenotypes in mice

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20% of Americans are at risk for environmental circadian rhythm disruptions (CRD) due to shift work. These individuals experience substantial negative health outcomes, but females are especially affected with greater vulnerability for substance use (SU) and adverse outcomes associated with pregnancy. These outcomes not only occur during pregnancy, but offspring are also affected at birth and later in life. Prenatal CRD (pCRD) in mice recapitulates these risks, increasing adverse pregnancy outcomes and altering behavior in adult offspring. However, it is unknown whether pCRD affects SU in mature offspring. C57Bl/6J dams were sham handled or disrupted, by reversing the light/dark cycle 4 times during gestation. Reward-related behaviors were measured in mature offspring. Contingency degradation was used to measure decision making. Mice were trained to respond on two levers for food, then the likelihood that one of those levers will be reinforced was degraded. Another cohort was trained to respond for food before jugular catheterization. After recovery, mice were trained to respond on another lever for cocaine. Acquisition, the reinforcing and motivational properties of cocaine, extinction and cue-induced reinstatement were measured. Interestingly, females exposed to pCRD developed an anhedonic-like phenotype with decreased food self-administration, cocaine intake and reinforcing properties of cocaine. On the other hand, males showed a SU-like phenotype with increased higher order food self-administration and cocaine reinforcement. Furthermore, male pCRD mice maintained goal-directed decision making, responding more on a reinforced aperture, while female pCRD mice did not, indicating habit formation. Together these results suggest that male and female mice exposed to pCRD respond differently for rewarding outcomes. The SU-like phenotype in male pCRD mice is likely driven by increased reinforcement or enhanced reward sensitivity, while the anhedonic-like phenotype in pCRD females is likely driven by decreased goal sensitivity. By understanding how disrupted rhythms during pregnancy affect SU vulnerability in mature offspring, we can develop novel therapeutic approaches for SU in adults.Funding Source: DA039865 (McClung), DA046117 (DePoy)

Nucleus reuniens inactivation impairs performance on a delayed nonmatch to position task.

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Working memory is the ability to hold information online to guide behavior and is critical for navigating the environment. The rat medial prefrontal cortex (mPFC), the dorsal hippocampus (dHPC) and their functional connection, are required for working memory. Since there is no direct projection between the mPFC and dHPC, the thalamic nucleus reuniens (RE) is a likely intermediary between these structures due to its anatomical connections and role in working memory. Here we aimed to determine if RE is required for performance in a delayed nonmatch to position (DNMTP) task. This task consists of a 1) sample phase (one lever extended, e.g., right) 2) time delay (2-32s) and 3) a choice phase, initiated by nosepoke, where the opposite lever (i.e., nonmatch) from the sample phase must be pressed (e.g., left) in order to receive a reward. Failure to press the sample lever, nosepoke, or press the choice lever, resulted in a trial omission. Fischer F344 rats (n=6) were trained on the DNMTP task. Once criterion (80% correct, 50 trials, 2s delay) was met, rats underwent stereotaxic surgery for bilateral implantation of infusion guide cannulae into RE. Rats were then trained in the task with 4 distinct delays (last session: 4, 8, 16, 32 s). Rats then received bilateral intracerebral infusions of the GABAA agonist muscimol (500 nM, 0.1 ul) or equal volume saline infusion into the RE with the order of drug treatment counterbalanced across rats. We found that pharmacological inactivation of the RE led to overall decreased performance on the DNMTP task as indicated by a decrease in percent correct across delays. Specifically, we found a main significant effect of the drug treatment (muscimol vs saline) across all delays ($F(1,5) = 14.84$, $p = 0.01$) but no effect of delay ($F(1.631, 8.155) = 1.51$, $p=0.27$). Thus, transient inactivation of the RE impaired overall performance DNMTP in a delay independent manner. Ongoing studies are examining how pharmacological inactivation of the dHPC affects performance on the DNMTP task. Finally, we will use inhibitory chemogenetic manipulation to determine if the mPFC-RE and the RE-dHPC circuits are required for behavioral performance. Funding: R00DA042934-03/04 (NIDA), 03S1 (NIA); T34GM127154 (NIGMS)

DISSECTING THE ROLE OF THE SUBSTANTIA NIGRA PARS RETICULATA IN OPIOID ABUSE

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Opioids are highly addictive drugs whose misuse has greatly contributed to the national opioid epidemic. Abuse liability of opioids, including heroin, has traditionally been thought to derive from drug rewarding effects that involve GABA-mediated disinhibition of dopamine neurons in the ventral tegmental area (VTA). However, this hypothesis has been challenged by recent reports that the rewarding effects of opioids rely on stimulation of mu opioid receptors (MORs) in other brain regions. Notably, the substantia nigra reticulata (SNr), whose native neurons are rich in MORs, has been largely ignored in opioid addiction research. Using a highly sensitive RNAscope in situ hybridization technique we determined cell-type specific expression of Oprm1 mRNA, encoding MORs, in the midbrain. About 46% of SNr GABA neurons were found to express Oprm1 mRNA, which is in striking contrast to the VTA, where only 28% of GABA neurons express Oprm1 mRNA. Using transgenic and optogenetic approaches, we then determined the causal role of SNr GABA neurons in reward-related behaviors. Optogenetic inhibition of SNr GABA neurons produced rewarding effects in vGAT-cre mice, as assessed by intracranial self-stimulation and real-time place preference. We also found that in vGAT-cre mice, response-contingent optogenetic stimulation of SNr GABA neurons reduced heroin reward (as evidenced by compensatory increases in heroin self-administration rates) and reductions in drug-primed reinstatement of heroin seeking, suggesting a critical role for these neurons in opioid reward and relapse. These findings were corroborated by our additional findings that intra-SNr infusions of naloxonazine or naloxone (MOR antagonists) produced similar effects in rats, well beyond those seen with intra-VTA infusions. Our findings expand our understanding of the neurobiological mechanisms underlying opioid addiction, pointing to SNr GABAergic neurons as a key player in some aspects of heroin-related behaviors. Importantly, our findings also redefine the primary function of the SNr, which has traditionally been thought to be restricted to motor processes.

Aging impairs contextual fear memory extinction retrieval and long-term potentiation in the basolateral amygdala.

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Aging is accompanied by a decline in several cognitive domains which can be exacerbated by emotional affective disorders such as anxiety, depression, and post-traumatic stress disorder (PTSD). One cognitive domain negatively impacted by aging is cognitive flexibility, which is critical in the ability to suppress old information (extinction) and retain new information (updating). Indeed, an inability to extinguish negative emotional memories can be detrimental to the overall quality of life in old age. While much of the work on cognitive aging has focused on memory function and cortical contributions to its decline, considerably less is known regarding the functional decline underpinning emotional-affective disorders in aging. Prior studies have shown the basolateral amygdala (BLA) plays a central role in the acquisition and extinction of negative emotional memories (i.e., fear memories) and the persistent overgeneralization of such events resulting in maladaptive responses. The current study used behavioral and electrophysiological approaches in young and aged rats to define the neural mechanisms underlying emotional affective impairments in aging.Young (6 mo) and aged (24 mo) Fisher344 rats were trained on a contextual fear conditioning paradigm, and at least 3 weeks post-testing, rats were sacrificed for ex-vivo slice electrophysiology to record extracellular field potentials from the basolateral amygdala. During fear acquisition, aging increased the magnitude of freezing behavior. While aged rats were not extinction impaired, aging did impair extinction memory retrieval after a 24-hour delay period. Additionally, the magnitude of fear renewal to context A was larger in aged rats. These impairments in aged rats may be due to attenuated LTP magnitude. These data suggest amygdala dysfunction as a function of aging may underlie an increase in the incidence of emotional affective disorders later in life.Support: Interdisciplinary Training in Pathobiology and Rehabilitation Medicine 2T32HD071866-06 to CMH and R01AG066489 to LLM.

Retrieval spikes: a dendritic mechanism for retrieval-dependent memory consolidation

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Retrieval-dependent processing of a new memory improve its long-term retention. To study neuronal mechanisms that mediate the effects of early retrieval on memory strength, we used a modified protocol of the classical fear conditioning paradigm, where presentation of a tone shortly after tone-shock pairing improved long-term memory retention. We presented such a reminder after a tone-shock pairing to head-restrained mice, while imaging neuronal calcium activity in their primary motor cortex, a region required for the acquisition of conditioned freezing. We found that reminders induced a substantial increase in calcium spike generation in apical dendrites of layer 5 pyramidal neurons, the main output cells of the cortex. However, the potency of reminders to evoke calcium spikes was transient, apparent within a time window shorter than 30 minutes. calcium activity during reminder-evoked dendritic spikes correlated with changes in postsynaptic spines: spines that were the most active during spiking underwent long-lasting weakening. To test the importance of reminder-evoked dendritic Calcium spikes, we optogenetically inhibited CaMKII, a master regulator of plasticity. Notably, CaMKII inhibition disrupted spine weakening, spike transience, and conditioned freezing, only when applied shortly after reminder-induced spikes. We describe a transient consolidatory process, promoted by reminders and mediated by dendritic spikes.

Estrous cycle-dependent modulation of stress-induced reinstatement of reward seeking in female mice

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Rates of alcohol use disorders are rising in women, but the factors that drive relapse in women are poorly characterized. Women are particularly vulnerable to stress-induced craving, which may promote relapse. Intensity of drug craving also appears to be modulated across the menstrual cycle in women, suggesting that circulating hormones may play a role in susceptibility to relapse. The present study determined whether ethanol dependence promotes stress-induced reinstatement in female mice and assessed whether this is modulated by the estrous cycle. Further, as our previous work suggests that modulation of astrocytic signaling may reverse ethanol dependence-induced behavioral impairments in female mice, and astrocyte function is modulated by circulating hormones, we assessed the expression of astrocyte-specific proteins across the estrous cycle. Ethanol dependence was induced using chronic intermittent ethanol exposure via vapor inhalation. Mice were trained using a conditioned place preference task for a food reward followed by extinction training, forced swim stress, and assessment of reinstatement. Forced swim stress facilitated reinstatement in both nondependent and dependent female mice and the magnitude of reinstatement was not different between the two groups. Reinstatement was attenuated during the diestrus phase of the estrous cycle in both nondependent and dependent mice. As diestrus is associated with increased levels of circulating progesterone in mice, a separate group of mice received a subcutaneous injection of progesterone (5mg/kg) or sesame oil 30 minutes prior to assessment of reinstatement. Progesterone administration significantly attenuated reinstatement compared to vehicle administration. A third group of female mice were sacrificed in each phase of the estrous cycle for assessment of the astrocytic marker, GFAP. GFAP immunoreactivity was significantly reduced in the nucleus accumbens and basolateral amygdala during diestrus. Together, these data suggest that relapse-like behavior may be modulated by the estrous cycle and that progesterone signaling may be a promising target for reducing relapse-like behavior in women. Work supported by AA027629 (JMB) and the Hartwell Foundation Postdoctoral Fellowship (LLG).

Neuronal ensembles in the ventromedial prefrontal cortex mediate initial oxycodone seeking and maintenance of oxycodone self-administration

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Oxycodone is a highly addictive opioid analgesic that has contributed to the current opioid epidemic. In human and animal models, drug-paired cues induce craving, leading to relapse. Neuronal ensembles underlie the associations between drug rewards and associated stimuli. However, the role of ensembles in initial oxycodone learning and subsequent maintenance contributing to drug seeking remains to be determined. Here, we sought to determine the role of vmPFC neuronal ensembles in initial learning of oxycodone self-administration. We trained male and female transgenic Fos-LacZ rats to self-administer oxycodone for 3hr daily sessions until an acquisition criterion (30 active lever presses) was met. Rats then underwent a 30 min induction session wherein presses on the active lever re-expose them to oxycodone paired cues without drug delivery in order to reactivate the initial acquisition memory. Next, we used Daun02 to selectively inactivate vmPFC Fos expressing ensembles associated with recall of initial oxycodone learning. We found that Daun02 compared to vehicle reduced oxycodone-seeking after initial acquisition of oxycodone self-administration. Next, we sought to determine if vmPFC neuronal ensembles also mediate oxycodone seeking pertinent to stable maintenance of oxycodone self-administration. We trained male and female transgenic Fos-LacZ rats to self-administer oxycodone for 3hr daily sessions under an FR1, FR2 and FR3 schedule of reinforcement to demonstrate stable oxycodone reward under increased effort to self-administer. We then put rats through 1 week of home-cage abstinence to control for acute withdrawal effects prior to the 30 min induction test to reactivate the recall of oxycodone self-administration. We found that Daun02 compared to vehicle attenuated oxycodone seeking relevant to maintenance of oxycodone self-administration. These results suggest that vmPFC neuronal ensembles are formed during initial learning of oxycodone self-administration and required for the maintenance and expression of oxycodone seeking. These findings may help uncover the neural underpinnings of oxycodone relapse. Funding source: NIDA Grant No. 4R00DA042102-02 awarded to BW and PA-18-906 awarded to CG

The effects of sex in Fischer F344 rats on a delayed non-match to position task.

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Working memory refers to the online retention of a small amount of information for a brief period of time. It is the storage of information that is not necessarily available in the immediate environment and can be used in the execution of cognitive tasks. Patients with Alzheimer's disease (AD) show working memory impairments during the early stages of the disease. Critically, there are sex differences in the rate of cognitive decline in AD. Cognitive decline is higher in women and AD is more prevalent in women compared to men. Addressing underlying sex differences is important for the development of effective therapeutics. In this study, we tested working memory in male (n=8) and female (n=8) Fischer F344 rats using a delayed nonmatch to position (DNMTP) task. The DNMTP task consisted of three phases; sample, delay, and choice phase. During the sample phase, the house light was illuminated and one of two levers (e.g. right) extended into the chamber. The rats were required to press the lever for a reward, which then initiated the delay phase. During the delay, all lights extinguished and the levers retracted. After the delay, the house light illuminated and the rats were required to nosepoke to initiate the choice phase. During the choice phase, both levers inserted into the chamber, and the rats were required to press the opposite lever (i.e., nonmatch) from the sample phase (e.g., left) to obtain a reward. In the final phases of the task, there were 4 delays (2-32 seconds) intermixed pseudorandomly with 15 trials/delay. The percent correct for each sex were compared across different delays. There was no significant difference in the DNMTP task between male and female rats during shorter delays as shown by a two-way repeated-measures ANOVA with sex and delay (2,4,8,16 s) as factors ($F(1, 28)=0.88$, $p=0.36$). However, a significant difference emerged during longer delays (4, 6, 12, 24 s) with males performing better than females ($F(1, 28)=4.87$, $p=0.036$). We are currently examining longer delay sessions (4,8,16, 32 s) and if we observe working memory impairments in transgenic rats (TgF344-AD) on this Fischer 344 background strain developed to recapitulate AD pathology in both sexes.Funding: R00DA042934-03/04 (NIDA), 03S1 (NIA); T34GM127154 (NIGMS)

Role of central acetylcholine release in the regulation of locomotion circuits.

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Impairment in cholinergic neurotransmission is associated with normal and pathological aging, making cholinergic release a subject of high interest to human biology. However, the precise role of changes in central acetylcholine release in mediating behaviors, such as locomotion, has not been fully elucidated. The vesicular acetylcholine transporter (VAChT) is responsible for the packaging of acetylcholine for exocytotic release. A complete loss of VAChT function is lethal, while severe mutations can cause decreased locomotive performance in Drosophila. However, the mechanism through which VAChT mediates vesicular release beyond its role in filling synaptic vesicles with acetylcholine is not fully understood. Here, we hypothesize that deficits in VAChT function can be rescued by a genetic increase in VAChT function, which can be identified by a shift in the rate of locomotion towards normal. In order to rescue the locomotion deficit seen in Vacht mutants, a wildtype copy of the VAChT gene was expressed in different Vacht mutant backgrounds. Here we report that the expression of a wildtype copy of VAChT rescues baseline locomotion and we compared its effect in two types of locomotion in Drosophila. Moreover, we present results regarding the effect of the wildtype Vacht rescue on the different Vacht mutant alleles. Our results demonstrate that genetic interventions are capable of ameliorating deficits caused by a loss of VAChT function. Together, these studies could pave way for future strategies to treat behavioral deficits associated with altered cholinergic signaling.We acknowledge support by the Delaware INBRE program, with a grant from the National Institute of General Medical Sciences - NIGMS (P20 GM103446) from the National Institute of Health and the State of Delaware. We also acknowledge the support of M. Harrington with a grant from the National Institute of General Medical Sciences- NIGMS (1P20GM103653-01A) from the National Institute of Health.

Impaired biconditional association ability in aged female Alzheimer’s disease (Tg-F344 AD) rat model

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There is a profound need to generate therapeutic strategies combating Alzheimer's disease (AD), as impaired cognitive faculties significantly impact the independence and quality of life for the 5.8 million Americans currently living with AD. Model organisms play a vital role in the search for such interventions. Thus, we utilized the transgenic Tg-F344 AD rat model, which recapitulates many of the histological and phenotypic hallmarks of AD in humans, to begin to characterize the role of age and metabolism in AD-related cognitive decline. Previously, these animals have been shown to have impaired performance on some classic measures of â€˜simple' types of memory at various ages, including object recognition memory, reversal learning and T-maze performance. In this study, we utilized a more complicated biconditional association task (BAT) that is capable of detecting age-related cognitive impairment earlier than spatial learning paradigms to investigate how age and metabolism intersect with the transgenic genotype in aged (20+ months) female rats. This behavioral paradigm not only relies on several brain regions within the medial temporal lobe and prefrontal cortex, which are traditionally affected by both aging and AD, but also requires interaction amongst these regions to remain intact. The incorporation of a spatial navigation task relying on working memory into an object recognition paradigm results in significantly poorer performance in AD rats relative to wild type (WT) counterparts (n = 5/group, p< 0.01). Furthermore, this effect of genotype was also apparent when an object-in-place rule was incorporated. However, simple object discrimination did not differ across groups (p = 0.51), demonstrating that this effect is likely from a cognitive, rather than physical, impairment. Furthermore, poorer performance on cognitive tasks correlate with increased body fat percentage and altered weight loss in response to stress. These data validate the Tg-F344 AD rat model as an appropriate model to investigate potential therapies to treat AD-related impairments in several cognitive domains.Funding: NIH NIA (R01AG054538 & P30 AG050886 to TWB & CSC and 1R01AG066489 to LLM) and NICHD (T32HD071866 to ARH)

Early impairments of fear memory extinction and amygdala plasticity in the TgF344AD rat model of Alzheimer’s disease are distinct from impairments in non-pathological aging.

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Alzheimer’s disease (AD) is accompanied by cognitive impairments affecting several memory domains. One such domain, emotional memory, is supported by a vast network of medial temporal lobe structures which are negatively impacted in AD. Human studies suggest the inability to acquire aversive emotional (fear) memories may be an early sign of AD that occurs prior to measurable cognitive impairments. The transgenic Fischer344 AD (TgF344AD) rat model recapitulates many aspects of human AD. The current study used behavioral and electrophysiological approaches in TgF344AD rats to define the contributions of genotype and age to fear memory impairments in AD. Young adult (YA, 6 mo) and middle-aged (MA, 14 mo) non-Tg (nTg) and TgF344AD (Tg) rats were trained on a contextual fear extinction paradigm and at least 3 weeks post-testing, rats were sacrificed for ex-vivo slice electrophysiology to record extracellular field potentials from the basolateral amygdala.In YA adult rats, there extinction impairments that carried over to retrieval and renewal testing. These extinction impairments in YA Tg rats may be due to increased strength of basal transmission, synaptically driven hyperexcitability, and attenuated LTP magnitude. In MA nTg rats, there were acquisition and extinction impairments relative to young nTg rats. Attenuated LTP magnitude was observed in MA nTg rats relative to nTg. Interestingly, MA Tg rats showed an enhanced ability to extinguish fear expression to the CS relative to MA nTg. However, MA Tg rats continued to show impairments in retrieval and renewal. Enhanced extinction in MA Tg rats may be associated with BLA compensatory mechanisms, as MA Tg rats showed attenuated field peak amplitudes, while having greater hyperexcitability, with no difference in LTP relative to MA nTg rats. These data recapitulate early deficits in human AD patients and further suggest deficits in AD manifest distinctly from nonpathological aging.Support: Interdisciplinary Training in Pathobiology and Rehabilitation Medicine 2T32HD071866-06 to CMH and R01AG066489 to LLM.

Neuroactivational and behavioral correlates of psychosocial stress-induced cocaine seeking in rats

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Risk of cocaine relapse can persist even despite prolonged abstinence. Exposure to psychosocial stress is thought to be a major contributor to cocaine relapse, yet the neural circuitry by which it induces drug seeking has not been thoroughly explored. This study aimed to identify brain regions that are selectively activated during psychosocial stress-induced cocaine seeking in rats. Adult male and female Long-Evans rats were trained to self-administer cocaine (0.5 mg/kg/inf i.v.) in 2-h daily operant-behavioral sessions for 20 d. On days 11, 14, 17, and 20, a discrete tactile cue was presented in the operant chamber that signaled impending social stress (social defeat, n=16), nonsocial stress (footshock, n=12), or a no-stress control condition (n=12) immediately after the session. Beginning on day 21, animals underwent extinction training during which lever-presses were not reinforced. Once responding was extinguished, rats were re-exposed to the cue that signaled their assigned stress/no-stress stimulus and cocaine-seeking was measured for 2 h under extinction conditions. Immediately after this reinstatement test, animals were sacrificed and brains collected and processed for c-Fos expression. When all groups were combined, cocaine-seeking magnitude was found to be positively correlated with neural activity in several brain areas, including medial prefrontal cortex (mPFC), paraventricular nucleus of the hypothalamus, and the anterior periaqueductal gray (aPAG). Interestingly, activity within different subdomains of the aPAG varied as a function of the stressor employed. Specifically, activation of the dorsal aPAG correlated with footshock-associated cocaine seeking whereas activation of the lateral aPAG correlated with psychosocial stress-associated cocaine seeking. Finally, activity in these aPAG subdomains were differentially correlated with activation in other brain areas. These studies suggest that psychosocial stress may trigger cocaine seeking via a unique neural network that involves the lateral aPAG

Prefrontal-hippocampal-thalamic circuit function during working memory task learning in a SETD1A deficient model of schizophrenia risk

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Working memory deficits are ubiquitous in schizophrenia. In rodents, neural synchrony between the ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC) is critical for working memory. Although vHPC-mPFC projections are unidirectional, the thalamic nucleus reuniens (Re) sends reciprocal connections to both the mPFC and vHPC, supports vHPC-mPFC synchrony, and is also necessary for successful working memory performance. To examine potential working memory-related prefrontal-hippocampal-thalamic circuit dysfunction associated with schizophrenia, we assessed neural synchrony and working memory performance in mice carrying a heterozygous loss-of-function mutation in the SETD1A gene. In human subjects, heterozygous null mutations of SETD1A definitively predispose individuals to the disease. Therefore, these studies may lead to the development of hypotheses regarding the pathophysiology of neural circuit abnormalities in individuals with SETD1A mutations and idiopathic schizophrenia.

Graph theoretical analysis of the default mode network in healthy older adults and adults with Mild Cognitive Impairment and Alzheimer’s Disease: an fMRI project.

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The default mode network (DMN) is a functionally interconnected group of brain regions that are active during resting states when an individual is not engaged in any cognitive task. Changes in DMN activity have been linked to memory decline in individuals with Mild Cognitive Impairment (MCI) and Alzheimer's Disease (AD). However, it is still unknown how AD preferentially targets regions of the DMN. Few studies have examined both structural and functional data from the same individuals. The current study addresses this gap by conducting multivariate analyses of both structural and functional brain scans from a large sample size of individuals. Specifically, we used graph theory analysis, which examines relationships between brain regions by representing them mathematically as a network of nodes and edges. Structural and functional neuroimaging data were obtained from the OASIS-3 dataset and include scans from 102 healthy controls and 102 MCI and AD patients. Based on previous research, 87 structural gray matter regions of interest were defined for the structural network analysis and 160 spherical regions of interest (ROIs) were defined for the functional analysis. Graph theoretical analysis was conducted using the Brain Connectivity Toolbox (BCT) in MATLAB. For the large network structural analysis, graph measures were similar to a prior finding; however, some differences were seen at the sub-network scale. Specifically, Cluster 2 showed changes in clustering coefficient that suggest a decrease in network efficiency (p=0.0017). This suggests that some structural clusters are more susceptible to the effects of AD. Functional analysis results showed significant difference for the modularity measure (p=0.02), similar to a prior finding. These findings suggest that MCI and AD alter functional brain networks. Specifically, the decrease in modularity points to less specialization of processing and decreased efficiency in impaired individuals. The large sample size and incorporation of both structural and functional data allow this study to uniquely demonstrate how brain connectivity is impacted by MCI and AD. Further investigation may examine specific nodes within the network, such as the hippocampus, which may show more disease-related changes.

Opioid withdrawal disrupts VTA inhibitory plasticity and produces negative affect via nitric oxide signaling

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Opioid use disorder is partially driven by a negative affective state during abstinence that extends long after physical withdrawal symptoms subside. Opioid replacement therapy alleviates negative affect, but it is limited by social stigma and abuse liability, and opioid antagonism further increases negative affect. A critical avenue for opioid use disorder research is to discover a non-rewarding drug that addresses affect specifically. To that end, we first assessed negative affect after chronic opioid administration using the tail suspension test. Male and female mice were injected with morphine (10 mg/kg) twice daily for 5 days and then had 7 days of withdrawal. Mice that received morphine exhibited more immobility during tail suspension compared to saline-injected controls, indicating increased negative affect. To investigate the mechanism for this behavioral effect, we next assessed plasticity at inhibitory ventral tegmental area synapses, which are capable of mediating both reward and aversion. In slices from drug-naive mice, high-frequency stimulation potentiated inhibitory post-synaptic activity (LTPGABA) on dopamine neurons. However, LTPGABA was disrupted in morphine-treated mice. The mechanism of LTPGABA is nitric oxide-dependent. We reasoned that a drug that acts on nitric oxide synthase might reverse abnormal behavior if the synaptic change underlies withdrawal-induced negative affect. Therefore, we treated mice with a nitric oxide synthase inhibitor (L-NAME, 1 mg/ml in drinking water) during withdrawal. Mice that received L-NAME exhibited less immobility during tail suspension compared to controls. Collectively, these data indicate that the nitric oxide pathway is a potential target to treat patients with opioid use disorder.

High-throughput phenotyping and genetic mapping of fibroblast rhythms and its correlation with cocaine addiction in genetically diverse mice.

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Substance abuse is associated with circadian disruption which increases the vulnerability to drug addiction. Circadian clocks regulate reward-related pathways, but the genetic mechanisms underlying this mutual interaction is not fully understood. Diversity Outbred (DO) and Collaborative Cross (CC) mice are powerful tools for examining the genetics of complex traits because they have high genetic and phenotypic diversity by genetic combinations of their eight founders including 5 common-inbred and 3 wild-derived strains. To understand the genetic link between circadian rhythms and addiction, we performed high-throughput phenotyping of circadian rhythms and cocaine addiction-related behaviors using DO, CC and their founders. We observed significant strain by sex effects in period and amplitude of fibroblast cellular rhythms among founders with strong heritability. Extreme phenotypic differences were observed between A/J and CAST/EiJ in cellular and behavioral rhythms: the longest period in A/J and the shortest period in CAST/EiJ mice. Our preliminary correlation analysis suggests that the founder with longer periods and with reduced amplitude displayed more relapse-like and drug-seeking behaviors in cocaine self-administration (IVSA) respectively. In CC strains, CC004 displayed significantly higher addiction-related behaviors in cocaine-sensitization and IVSA relative to CC041. CC004 also exhibited a longer circadian period and lower amplitude in cellular and behavioral rhythms relative to CC041 mice. Furthermore, we measured cellular rhythms in 329 DO mice and they displayed more circadian phenotypic variability than the founders: 80% of founders compared to only 25% of DO mice had periods of ~24 h. We also identified several quantitative trait loci for cellular rhythms. Collectively, our findings demonstrate that genetic diversity contributes to phenotypic variability in circadian rhythms and prove that high-throughput screening of fibroblast rhythms in DO mice is powerful for high-precision genetic mapping.

Mild traumatic brain injury produces transient increases in risk taking behavior in female rodents.

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Mild traumatic brain injury (mTBI) is the most common form of TBI. Many individuals, such as athletes, often experience repetitive mTBIs over the course of their athletic careers. Consequences of mTBIs include impairments to higher order cognitive processes such as decision making. Risk-based decisions involve choices that have probabilistic outcomes. Increased risk taking behavior has been reported for TBI patients following a single TBI event; however, little information is available regarding the effects of repetitive mTBIs on risked-based decision making. Here, we examined how single and repetitive mTBI affects risk-based decision making in rodents using a well-established probabilistic discounting task (PDT). Age-matched male and female rats were trained on the PDT and then exposed to either a single or a series of three closed head control cortical impact (CH-CCI) injuries over the course of one week. Rats then returned to the PDT for testing for four weeks. We observed no changes in risky choice behavior across all four weeks of testing in male rats following either single or repetitive mTBI. In contrast, mTBIs, specifically repetitive injuries, increased risky choice behavior in female rats at one and two weeks post injury. Increased risky choice behavior was more prominent two weeks post injury with effects being driven by a reduced sensitivity to non-rewarded outcomes following a risk-based decision, demonstrated by a decrease in lose-shift responses. When evaluated three and four weeks post injury, choice behavior was no longer significantly different from uninjured animals indicating that the observed effects of mTBIs in female rats are transient. These results indicate that females are more susceptible to changes in risk-based decision making following repetitive mTBIs in comparison to males of the same age. Overall, when used together, the CH-CCI model of repetitive mTBI and the PDT are useful tools for experimentally evaluating and differentiating the effects of head injury on male vs female subjects.Funding sources: New Jersey Commission on Brain Injury Research CBIR20PIL004 (Navarra) and CBIR19IRG025 (Waterhouse)

Lethal and toxic effects of methamphetamine, dimethylone and pentylone

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There has been an increase in the use and abuse of synthetic psychoactive cathinones (SPCs) over the past two decades. With the growing popularity of SPCs, there has also been a growing concern regarding their toxicity. SPCs have structural similarities to amphetamines, but their pharmacological attributes have shown some differences. Some clinical studies have shown that SPCs have adverse effects on cardiac function that may contribute to their propensity for acute overdose. It is also important to note that SPCs are usually used in a â€œclub-likeâ€• environment where the ambient temperature is higher than normal. This fact raises concerns that toxicity may be increased when SPCs are used under higher ambient temperature conditions. In this study, 5 days-post-fertilization (dpf) larval zebrafish were used to assess the effects of SPCs on heart rate under normothermic and hyperthermic conditions. The SPCs used in this study were dimethylone (DiMETH) and pentylone (PENT). Methamphetamine (METH) was used a positive control because it is well-known to show temperature-dependent toxicity. Methods: Lethality, cardiotoxicity (heart rate) and hepatic toxicity (whole body ammonia) of synthetic cathinones were examined in 5dpf zebrafish after a 5h exposure under normothermic (28.0 degrees C) and hyperthermic (32 - 34 degrees C) conditions METH, DiMETH and PENT (1microM ~ 10 mM) were administered via direct immersion. Results: As expected, hyperthermic conditions potentiated METH lethality and cardiotoxicity. A similar temperature dependency was shown for both DiMETH and PENT. More, limited effects on hepatic toxicity were seen. Conclusion: These studies show that larval zebrafish can be used to study the effects of ambient temperature on the lethal and toxic effects of amphetamines and cathinones. Moreover, they show that high temperature conditions greatly increase the lethal and toxic effects of cathinones, which have been shown to have somewhat limited lethal and toxic effects under normothermic conditions in mice. Therefore, to understand the potential of cathinones for adverse effects and overdose it will be important to examined them under hyperthermic conditions. Funding: NIH DA045350 (FSH)

Sex-Specific Lasting Impacts of Adolescent Binge Alcohol Exposure on Behavioral and Metabolic Profiles in C57BL/6J mice.

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Given the adverse effects of adolescent binge alcohol consumption in humans, the present work explores the lasting behavioral and metabolic impacts following adolescent binge ethanol exposure in male and female C57BL/6J mice. The behavioral tests were mapped alongside with metabolic analysis of serum and fecal samples. Male and female mice were exposed to binge ethanol exposure using intermittent vapor inhalation that included 2 days of exposure followed by 2 days of abstinence, repeated 4 times from postnatal day (PND) 28 to PND 41. Mice were tested for affective behaviors using the open field test (OFT), the light/dark test (LDT), and the tail suspension test (TST) from PND 49-53 and every 2 weeks thereafter until PND 92-95. Serum samples were collected 24 hours after the last exposure (PND 42) and fecal samples were collected for metabolomic analysis during the first and last behavior test (PND 49-53 and 92-95, respectively). Metabolic data was collected using nuclear magnetic resonance (NMR) and analyzed using principal component analysis (PCA). Analysis of serum samples showed that male mice exposed to ethanol had increased glucose and decreased amino acid levels compared to air-exposed controls. One week following the last ethanol exposure cycles, the male mice showed similar differences in their metabolomic profiles using fecal samples. No metabolic changes were observed between female groups. In the LDT, ethanol-exposed male mice show higher affinity for the light compartment during the first two behavioral measurements (PND 49-53 and 63-67). No clear differences in immobility were found for any of the groups during TST. Finally, during OFT (PND 92-95), using the center zone as a measure of anxiety-like behavior, ethanol-exposed mice showed more time spent and more entries into the center zone. No differences were found for distance traveled or rearing. We plan to analyze substrate correlation with behavioral trends following binge alcohol exposure during adolescence. Together, these data show that binge alcohol exposure during adolescence impacts metabolic processes and some behavioral measures, more profoundly in males, but these effects may not be long-lasting. Funding: KL2TR002490 and NC A&T CEEI Seed Grant

Social transmission of maternal behaviors through observation

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Oxytocin is a peptide hormone important for maternal physiology and social behaviors (Jurek & Neumann Physiol Rev 2018). One behavior influenced by central oxytocin signaling in rodents is pup retrieval (Marlin et al. Nature 2015; Scott et al. Nature 2015; Schiavo et al. Nature 2020). When mouse pups are out of the nest, they emit vocalizations that prompt dams to fetch the pup back to the nest. Virgin females do not initially retrieve pups, but can learn to retrieve after several days of co-housing with an experienced mother and pups in a manner that may involve visual observational learning of dams interacting with the nest and pups (Carcea et al. BioRxiv 2019). To explore what aspects of maternal behavior are socially transmitted, we showed audio-visual movies to head-fixed awake virgin female mice that initially did not retrieve pups. This head-fixed approach allows us to manipulate the sensory stimuli presented to the virgins, in order to explore what aspects of sensory experience trigger oxytocin release and lead to alloparenting behaviors. We found that head-fixed virgins exposed to audio-visual movies of maternal retrievals (N=18) expressed alloparenting faster than virgins presented with dark backgrounds (N=21,p=0.030). Alternatively, virgins presented with videos of maternal mistakes (out of the nest retrievals; N=4) or non-maternal behaviors (video of conspecifics; N=14) also rarely expressed alloparenting behavior. We optogenetically suppressed oxytocin neuron activity during exposure to movies of dams retrieving pups, and found that this manipulation prevented the enhancement of alloparenting by maternal video playback (N=4, p=0.031). These results demonstrate that maternal behavior in mice can be socially transmitted, and that the mouse visual system provides input to the oxytocin neurons for enhancement of alloparenting. Funding: HHMI Gilliam Fellowship, NSF GRFP and NIH

Loss of cerebellar output neurons during development is associated with learning deficits.

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Developmental perturbations in the cerebellum lead to long-lasting social and cognitive deficits, but we do not understand how the cerebellum regulates the assembly and function of forebrain circuits that modulate non-motor behavior. The excitatory cerebellar nuclei (eCN) in the three bilateral CN are the major cerebellar output neurons and connect to the neocortex via the thalamus. We recently showed that mutation of the mouse homeobox genes Engrailed 1 and 2 in eCN using Atoh1-Cre leads to embryonic loss of the posterior medial and posterior intermediate eCN and their presynaptic Purkinje cells but adult cerebellar cytoarchitecture is remarkably normal. We showed that these mutants have shorter stride length and impaired rotarod performance (Willett, Bayin, Lee, Krishnamurthy, Wojcinski et al., eLife, 2019), and here we observed hypolocomotion (open field) which may relate to the stride defects. Furthemore, we tested social, cognitive and affective behaviors in male mutants compared to littermate controls and surprinsingly only observed a learning deficit (water Y-maze). Retrograde (Fluoro-Ruby) tracing of two thalamic nuclei that control cognition (mediodorsal and parafascicular) showed reduced labeling only in the posterior medial and posterior intermediate eCN in mutants. Anterograde (BDA) tracing of the remaining mutant medial and intermediate eCN revealed no ectopic projections to the thalamus. Given the role of the striatum in rotarod and water Y-maze performance and that the eCN connect to the striatum through the mediodorsal and parafascicular nuclei, we are testing whether aberrant thalamo-cortico-striatal circuits in these mutants underlie the behavioral deficits. This work was supported by R37MH085726 (ALJ); T32HD060600 (ASL); FCLC Dean's Undergraduate Research Grant (AG).

Dorsal and ventral hippocampus remapping in response to novel experiences.

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In both humans and rats, the hippocampus is important for memory and navigation. Individual hippocampal neurons are spatially tuned, and fire in specific physical locations. Together, these "place cells" provide a representation or map of an environment. However, if the environment or behavioral context is altered, a place cell can change its firing pattern or "remap". The hippocampus is a long structure with multiple sub-regions of diverse connections. Much is known regarding place cell activity in the dorsal region of the hippocampus since these are close to the surface of the brain and relatively accessible. Less is known about ventral hippocampal place cell activity located much deeper in the brain. We have constructed a 64-channel hyperdrive, with 16 independently moveable tetrodes targeting both dorsal and ventral regions. Firing characteristics were determined while we recorded single units simultaneously from the dorsal and ventral hippocampus as rats performed a familiar/well-learned task (traversing a linear track). After the hippocampal representation of the familiar task was determined, one of the two arms was rotated to a novel position. We measured how the spatial tuning of these neurons were modified when rats experienced this novel spatial environment. Similarly, we compared the hippocampal representation of the familiar task with novel olfactory experiences (female bedding and coyote urine). We will determine dorsal and ventral hippocampal representations of a familiar maze configuration, the development of a new representation, and the degree to which the developed representation is stable. Specifically, overall firing rates and place field characteristics will be examined. Taken together, these data provide insight into the relationship between different regions of the hippocampus during the processing of a novel experience. This research was funded in part by The Crandall-Cordero Fellowship, Peter and Carmen Lucia Black Foundation, CT Institute for the Brain and Cognitive Sciences, UConn SLAC, and UConn IDEA Grant.

Aberrant innate behavioral responses to predator odor in mice genetically deficient for dopamine beta-hydroxylase (Dbh).

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The catecholamines dopamine (DA) and norepinephrine (NE) are classically associated with reinforcement and aversion, respectively. DAergic neurons in the midbrain reinforce approach and appetitive behaviors through their projections to the medial prefrontal cortex (mPFC), nucleus accumbens, and amygdala, which are involved in voluntary action and reward evaluation. While noradrenergic projections from the locus coeruleus and other brainstem nuclei partially overlap with those of DAergic neurons in regions like the mPFC, amygdala, and hypothalamus, NE signaling within these regions is instead associated with avoidance and aversion. Importantly, a major difference between DA neurons and NE neurons is only the latter express Dbh, which converts DA to NE. We have shown that Dbh knockout (Dbh-/-) mice lack NE completely but produce excessive DA in their noradrenergic neurons, and also exhibit a striking absence of anxiety-like and repetitive behaviors in response to innate stressors like cage change or environmental novelty. Specifically, Dbh-/- mice failed to engage in typical nestlet shredding when placed in a new cage. When nestlets were infused with a novel, neutral odor (lavender), the control mice built nests and fell asleep in them, while Dbh-/- mice displayed their typical no-shredding phenotype and fell asleep without nests, as usual. Intriguingly, this no-shredding Dbh-/- phenotype was reversed when the nestlets were saturated with predator odor (bobcat urine); following this manipulation, Dbh-/- mice readily shredded the urine-soaked nestlets and fell asleep in them. By contrast, in response to the predator odor-treated nestlets, the NE-competent controls did not built nests and remained aroused and vigilant during the task. Moreover, Dbh-/- mice exhibited atypical active and passive coping responses to the predator odor during the first 10 min of the exposure. We plan to use pharmacology and activity-dependent mouse genetic tools to interrogate the neurochemical and circuit abnormalities associated with predator responses in Dbh-/- mice. These experiments will illuminate fundamental mechanisms by which catecholamines organize appropriate responses to innate stressors. Funding: NIH 2T32 NS 007480-20 to DL

A corticothalamic circuit for top-down inhibitory control of social motivation in male mice.

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Deficits in social motivation are common in many neuropsychiatric disorders such as depression, anxiety, and autism spectrum disorders. However, the neural mechanisms by which the brain regulates social behavior remain unclear. Previous research has shown that parvalbumin (PV) interneurons in the prefrontal cortex (PFC) are active during social behavior, and enhancing PV interneuron activity can rescue sociability deficits. However, a population of PFC neurons that may be inhibited by PV interneurons during social behavior has yet to be identified. In our study, we first tested whether neural connections between the PFC and the posterior paraventricular thalamus (pPVT) play an essential role in regulating social motivation using a chemogenetic approach. We found that chemogenetic activation, but not inhibition, of PFC-pPVT neurons abolishes social preference without affecting sociability. Moreover, this effect seems to be specific to male mice. Based on our finding, we hypothesize that this population of prefrontal neurons is selectively inhibited by activation of PV interneurons prior to an effective social interaction occurring. To test this hypothesis, we implement fiber photometry to assess changes in calcium activity in PFC-pPVT neurons during social interaction and in the future, will repeat this experiment while simultaneously inhibiting PV interneurons. We expect that our results will show evidence for a novel circuit mediating prefrontal top-down inhibitory control of social motivation. A mechanistic understanding of brain activity during social interaction will help develop better biomarkers for symptomology and better treatment options for social deficits. Acknowledgments: This study was supported by NIH R21MH110678, the NIH R21MH121836-01A1, and Pennsylvania Commonwealth 4100085747 (CURE 2020) to W. J. Gao; as well as the 2021 Dean Fellowship from Drexel University College of Medicine to N.R. Mack.

Dopamine correlates of habit versus goal-directed behavior in the ventral tegmental area

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Habitual learning would be mediated by a shift in reward seeking control from mesolimbic to nigrostriatal dopaminergic pathways. However, how midbrain dopamine (DA) neurons activity differentially signal habitual versus goal-directed performances remains largely unknown. To answer this question, we used TH-Cre transgenic rats, injected with a Cre dependent GCaMP6f virus into the VTA, and implanted with optic fiber above the injection site. The calcium photometry imaging allowed us to specifically measure and compare DA signal dynamics in two operant paradigms, namely the lever insertion fixed-ratio 5 (LI5) task and the lever retraction fixed-ratio 5 (LR5) task, promoting goal-directed or habitual behaviors, respectively. In these tasks, one of the cues - either the lever insertion or retraction - is relevant to signal reward (sucrose 20%) availability or delivery while the other cue is made irrelevant. We found that behavior quickly becomes automatic and habitual in the LR5 task in which the LR cue predicts immediate reward delivery. We further observed a rapid shift in the activation of DA VTA neurons from reward retrieval to the earlier LR cue, followed by a decrease in cue-related DA signal, across repeated trials, as rats develop habitual behavior. In contrast, in the LI5 task promoting goal-directed behavior, cue-induced DA activation remains relatively constant across trials and sessions, when reward availability is signaled by the LI cue, more distal from reward delivery. These results show manifest task differences in DA signaling that appear consistent with the difference in habitual versus goal-directed control of behavior.Supported by NIH R01DA035943.

The effect of methylone pre-exposure on MDMA, MDPV and fluoxetine-induced conditioned taste avoidance.

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The abuse potential of a drug is thought to be a balance of its rewarding/aversive effects, and several subject and experiential factors impact this affective balance. In this context, new psychoactive substances such as the synthetic cathinones are being examined to characterize their aversive and rewarding effects. As noted, preexposure to a drug has been reported to attenuate its own (and others') aversive effects, shifting the affective balance to reward. The present study assessed the effects of preexposure to methylone, a first-generation synthetic cathinone that serves as a substrate releaser for DA and 5-HT, on taste avoidance induced by the synthetic cathinone MDPV and the antidepressant fluoxetine that preferentially increase DA and 5-HT levels, respectively. Male Sprague-Dawley rats (n = 48) were exposed to vehicle or methylone (10 mg/kg) every 4th day for a total of five injections prior to taste avoidance conditioning in which a novel saccharin solution (1 g/L) was paired (five times) with MDPV (1.8 mg/kg) or fluoxetine (10 mg/kg). In vehicle pre-exposed animals, MDPV and fluoxetine induced conditioned taste avoidance relative to animals injected with vehicle during conditioning (p< 0.05). Methylone pre-exposure attenuated the avoidance induced by MDPV but had no effect on fluoxetine-induced avoidance (p< 0.05). The attenuation of the reuptake inhibitor MDPV's avoidance and the unaffected avoidance induced by the 5-HT reuptake inhibitor fluoxetine suggest that methylone's actions on DA likely mediate its (and MDPV's) aversive effects. These results suggest that drugs with common neurochemical substrates likely interact to impact abuse potential. The present study was supported by the Mellon Foundation (ALR) and the College of Arts and Sciences at American University (HNM).

Sex and genotypic differences in the genomic signatures of oral corticosterone in the dorsal and ventral hippocampus

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Dorsal and ventral hippocampus (HPC) are functionally distinct brain structures due to differences in their respective neuroanatomical connectivity and in the biological processes that they encode. These two hippocampal circuits also show anatomical sex differences in response to stress and display region-specific patterns of genes both at baseline and after pharmacological or environmental stimuli. We used RNA-sequencing to study differentially expressed genes (DEGs) in the dorsal (dHPC) and ventral HPC (vHPC) of male and female mice maintained on chronic oral corticosterone (CORT), a pharmacological method in neuroendocrinology that induces a blunted endocrine response to acute stress. Mice were wild-type (WT) or heterozygous for the brain-derived neurotrophic factor gene variant Val66Met (BDNF het-Met), a human SNP that increases susceptibility to stress. We found that CORT induced a greater number of DEGs in the vHPC compared to the dHPC regardless of sex and genotype, consistent with data showing that oral CORT largely impairs affective behavior. The gap between DEGs in the vHPC compared to the dHPC was more prominent in BDNF het-Met males than in WT males. CORT induced a lower number of DEGs in females than in males regardless of brain region and genotype. Unlike males, CORT induced more DEGs in the dHPC than in the vHPC in WT females and had a relatively minor effect in BDNF het-Met females. A significant number of DEGs were induced uniquely in either sex, brain region, or genotype. CORT-induced DEGs were grouped using GO that showed a majority of metabolic and neurotransmitter pathways in males and several pathways of immediate early genes that were unique to females. Together, these data demonstrate that exogenous CORT leads to discrete genomic signatures in the HPC associated with different biological processes as a function of sex, genotype, and hippocampal circuit.

Ventral Tegmental Area glutamate neurons mediate the non-associative consequences of traumatic stress.

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Exposure to uncontrollable stress is a risk factor for the development of a number of mood disorders, and has been shown to reduce social exploration and engagement. Glutamate neurons within the ventral tegmental area (VTA) are functionally important for signaling the presence of aversive or threatening stimuli. However, it is unknown how these neurons contribute to stress or social behaviors. This work establishes an operant mouse paradigm to evaluate the neuronal contribution of VTA glutamate neurons within the context of stressor controllability. Stressor controllability models how the psychological perception of control can be efficacious in blocking social and fear related deficits following stress experience. We used fiber photometry to measure VTA glutamate neuron activity using the genetically encoded fluorescent indicator GCaMP6m during escapable and inescapable stress conditions. VTA glutamate neuron activity was chemogenetically suppressed during uncontrollable stress using inhibitory designer receptors exclusively activated by designer drugs (DREADDs, hM4Di). Our results indicate that 1) inescapable stress (IS) results in reduced social exploration in male but not female mice, 2) control over shock termination, otherwise known as escapable stress (ES), is sufficient to block social exploration deficits, 3) VTA glutamate neuronal signaling (measured by GCaMP6m) is enhanced by both inescapable and escapable stress conditions, and 4) VTA glutamate neuron chemogenetic inhibition (via hM4Di activation) is sufficient to block social deficits and reduce generalized fear in the absence of behavioral control over the stressor. These results provide causal evidence that VTA glutamate neurons contribute to stressor outcomes and suggest that these neurons are capable of modulating social and fear deficits following stress. Funding Acknowledgement: Boettcher Foundation

The subfornical organ integrates with prefrontal cortical circuitry to regulate fear relevant to PTSD.

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PTSD associates with dysregulated fear processing. Most prior fear-related research has focused on assessing responses to externally evoked stressors. Yet, mounting evidence suggests PTSD patients are more sensitive to homeostatic stressors such as CO2 inhalation which triggers acidosis. CO2 inhalation evokes anxiety- and fear-associated defensive behaviors in clinical and preclinical models. Interestingly, pre-deployment CO2 sensitivity in veterans associates with later trauma-induced PTSD symptoms. This suggests CO2 sensitivity pre-dates development of PTSD and that investigating the relationship between homeostatic stressors like CO2 inhalation and externally evoked stressors may improve our understanding of PTSD vulnerability. We recently developed a mouse model examining the effect of CO2-inhalation on later PTSD relevant behaviors and found CO2 exposure increased later shock-evoked fear extinction deficits. These effects associated with reduced neuronal activation within the infralimbic (IL) cortex which plays a primary role in fear extinction suggesting a possible convergence of homeostatic CO2-sensing nodes and forebrain extinction circuits. We previously reported the subfornical organ (SFO) as a key homeostatic site regulating CO2-evoked fear. Here, we identified direct SFO projections to the IL by AAV tract tracing. Using an intersectional chemogenetic approach we tested the hypothesis that inhibiting SFO-IL projections during CO2-inhalation attenuates defensive responding during CO2-inhalation and delayed CO2-associated fear extinction deficits. We found DREADD mediated inhibition of the SFO-IL circuit during CO2 inhalation reduced CO2-evoked defensive behaviors. It also attenuated CO2 potentiation of contextual conditioned fear and improved extinction 1 week later. Importantly, SFO-IL inhibition did not impact SFO-related homeostatic responses such as water consumption, highlighting a specific role for this circuit in threat responding. Collectively, these data elucidate a novel SFO to IL projection that converges homeostatic threat sensing and long-term modulation of fear memory that may be relevant to understanding mechanisms underlying vulnerability to develop PTSD. Support: VA Grant BX001075; F32MH117913.

Changes in stress reactivity and stress-related behaviors following stress-induced escalation of cocaine intake.

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Stress is an important contributing factor to addiction and is problematic as stress is unavoidable in daily life. Addiction can be characterized by a loss of control over drug intake that is modeled by escalating patterns of drug self-administration (SA). We have shown that a stressor, footshock, administered daily at the time of SA induces an escalation of cocaine intake. This is likely due to long-lasting neuroplastic changes that also increases susceptibility to reinstatement of drug-seeking behavior. This may be explained by changes in stress reactivity as increased stress reactivity is associated with the severity of relapse. We hypothesize that repeated stress at the time of SA increases susceptibility to stress-induced reinstatement and alters stress-related behaviors and stress reactivity. Male rats were trained to SA cocaine or saline in 4 X 30 min SA sessions separated by 5-min drug-free periods. Some rats received shock in the SA chamber during the 5 min drug-free daily for 14 days. Rats were tested for reinstatement of drug-seeking behavior to a footshock stressor or yohimbine. A history of shock during SA resulted in increased stress-induced reinstatement. Rats were also tested for changes in affective (open field, elevated plus maze, marble burying), social, and cognitive (object location memory) behaviors. Rats were also tested for changes in stress reactivity in response to a heterotypic stressor (30-min restraint stress). Tail blood were taken during and after the restraint stress to asses changes in plasma corticosterone. Brains were collected 90 min after stressor initiation to measure stress-related changes in cFos expression in stress- and reward-related brain regions. Affective, social, and cognitive behaviors were altered depending on the rat's drug and/or stress history. The HPA axis corticosterone response to the restraint stress was also altered depending on the rat's drug and/or stress history. These data suggest that stress-induced neuroplastic changes occur that have long-lasting consequences to influence likelihood of reinstatement of drug-seeking behavior and the behavioral, hormonal, and neural response to stress. Funded by NIDA Grant K01DA045295 to Jayme McReynolds

Neurochemical Profile and Brain Electrical Activity Changes Associated with Cognitive Impairments (mouse model study)

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Integrity of neurotransmissions and their balanced interplay are crucial factors for proper functioning of neural circuits responsible for various behavioral stereotypes and patterns, emotional, motor activity and cognitive status. Deterioration of cholinergic and glutamatergic neurotransmissions is a pathognomic hallmark of Alzheimer's disease. Inhibitory neurotransmission weakening shifts excitatory/inhibitory (E/I) balance towards excitotoxicty, which also may be linked to dysregulation of monoamine neurotransmissions. Cognitive function disruption, particularly upon aging was associated with chronic stress. Moreover, chronic and repeated stress lead to acceleration of loss in cognitive performance in AD model animals, Abeta-peptide generation and exacerbation of amyloid plaques deposition and Tau hyperphosphorylation. Advantage of stress-resilient dominant (Dom) and stress-vulnerable submissive (Sub) mice model, allowed us to show a) difference in short- and long-term synaptic plasticity in Dom and Sub mice; b) innate, stress-exposure unrelated early cognitive impairment in working and reference memory domains in Sub mice; c) differential protein expression in the functional protein-protein interaction networks of synaptic transmission, circadian regulation and tyrosine protein kinase pathways; d) correlation between synapsin 2b and GluA1 protein levels diversity in Sub and Dom mice and early appearance of cognitive impairment; e) life expectancy difference in Sub and Dom mice markedly affected by chronic mild stress (CMS); f) Sub mice cognitive performance deterioration upon CMS, g) different response to cannabinoids. In this report, using advantage of Dom-Sub stress-resilience/vulnerability and 5xFAD Alzheimer's disease mice models, we show age-dependent and cognitive-status correlating changes in neurotransmitters (monoamines, glutamate and GABA) and their metabolites levels diversities and brain electrical activity patterns in Sub and 5xFAD mice vs their control counterparts.

A skin-to-brain circuit for social postures and sexual receptivity.

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Social touch is a critical component of both platonic and romantic relationships. Yet, the neurobiological mechanisms by which we perceive the rewarding valence of social touch, including how peripheral inputs engage oxytocin and dopamine circuitry to influence behavioral outputs, remain unclear. While C-tactile afferents are thought to convey the affective component of social touch in humans, analogous dorsal root ganglion neurons in mice have yet to be implicated in social behaviors. We demonstrate that a small population of DRG neurons marked by expression of Mrgprb4 are both necessary and sufficient for specific social behaviors and possibly for engaging neural circuits of social reward. Upon ablation of Mrgprb4-lineage neurons, female mice fail to develop lordotic sexual receptivity and less frequently exhibit back-lowering in response to cagemate contact, a social dorsiflexion we term the â€œconspecific crawlâ€• . In addition to being required for these social postures, the Mrgprb4-lineage neurons are, upon transdermal optogenetic activation, sufficient to induce a strikingly similar dorsiflexion in socially-isolated mice. This posture represents the first acute behavioral response to the optogenetic activation of social touch neurons, positioning social touch in the ranks with itch and pain for yielding a stereotyped, quantifiable motor output upon selective stimulation. The same optogenetic stimulus induces conditioned place preference, suggesting the posture reflects a response to a pleasant sensation, and that Mrgprb4-lineage neurons may activate reward circuitry. To test this hypothesis, we are using fiber photometry to measure dopamine release during optogenetic activation of Mrgprb4-lineage neurons in behaving mice. As female-female crawling behaviors are associated with oxytocin neural activity, we use the same approach to determine if the Mrgprb4-lineage neurons are sufficient to evoke calcium transients from oxytocin neurons. We conclude that Mrgprb4-lineage neurons are required to mediate touch-dependent social postures, sufficient to replicate them in socially isolated mice, and thus represent the first neurons of a potential skin-to-brain circuit for social postures and sexual receptivity. Funding: NRSA F31 NINDS

The impact of social stress on approach-avoidance behaviors

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Under approach-avoidance scenarios, healthy individuals balance their decisions towards the most favorable outcomes. However, individuals suffering from major depressive disorder (MDD) display deficits in these decision-making processes. Specifically, they show blunted approach towards rewards and excessive avoidance of previously aversive stimuli. Here, we adapted the platform-mediated avoidance (PMA) task developed in rats to assess decision-making under approach-avoidance conflict in mice previously exposed to chronic social defeat stress (CSDS), a well-validated model of MDD. Following CSDS, we classified the mice as resilient (RES) or susceptible (SUS) based on their social interaction profile (SUS mice show social avoidance whereas RES mice do not, F(2,61)=45.6, p<0.0001). Subsequently, we trained the mice in the PMA task whereby they learn to avoid a tone signaling a footshock at the cost of losing access to pressing for a saccharine-water reward. We recorded lever presses and time on platform as approach and avoidance learning, respectively. After ten days of acquisition (9 tone/shock pairings daily), we exposed the mice to four days of extinction (9 tones/no shock daily). While we did not observe significant differences in the acquisition of avoidance (F(2,61)=0.2, p=0.86) or lever pressing (F(2,61)=1.7, p=0.19) among groups, SUS mice show elevated levels of freezing (F(2,61)=4.2, p=0.02). Moreover, during extinction we found that RES mice show reduced time on the platform (F(2,61)=3.1, p=0.05) and increased lever pressing (F(2, 61)=6.5, p=0.003), suggesting facilitation of extinction learning. In contrast, SUS mice show elevated avoidance and blunted lever pressing. Together, our results indicate that RES mice balance their behavior towards approach when contingencies change (i.e., during extinction), whereas SUS mice balance their behavior towards avoidance. This is consistent with growing evidence suggesting that resilience is not the absence of susceptibility, but rather an active response to stress involving a unique behavioral phenotype which might reveal novel neurobiological markers to treat neuropsychiatric disorders such as MDD.FUNDING: NIMH

Identification of Novel Hedonic Hotspots in OFC, Insula, and Anterior Cingulate Cortex that Amplify 'Liking' and 'Wanting' for Sweet Reward.

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Dysregulation of brain mechanisms that control hedonic function can lead to various affective disorders such as depression, anhedonia, obesity, and binge-eating disorder. Hedonic function, or 'liking' can be objectively measured by pairing brain manipulations with the taste reactivity test, which assesses affective orofacial expressions elicited to various tastes, and which are homologous across various mammalian species, including humans and rodents. 'Liking' reactions are amplified by a network of brain hedonic hotspots, or circumscribed subregions in rat nucleus accumbens, ventral pallidum, orbitofrontal cortex (OFC), and insula when neurochemically stimulated. Beyond neurochemical triggers, the neuronal activation patterns and larger hedonic circuitry that generates affective 'liking'; reactions is not well understood. Here we present novel optogenetic evidence that neuronal stimulation of hedonic hotspots in rostromedial OFC and caudal insula double hedonic 'liking'; reactions to sweet sucrose, and are robust enough to suppress the aversive impact of a bitter quinine solution that is normally 'disgusting'. Photoexcitation of cortical hedonic hotspots can amplify 'wanting' for sweet reward, and even generate robust laser self-stimulation in the absence of reward. Importantly, the hedonic effects are anatomically restricted as the same manipulations in caudal OFC and anterior IC fail to amplify 'liking'; expressions, and even oppositely suppress hedonic reactions despite still generating robust 'wanting'; at some sites. Furthermore, we additionally identify the existence of a novel hedonic hotspot in caudal anterior cingulate cortex (ACC) never previously described. Our findings show that optogenetic stimulation of caudal ACC similarly doubles hedonic 'liking'; reactions in rats, and generates robust wanting; and laser real-time place preference. Finally, we measure Fos protein expression in hedonic hotspot output circuitry. Our findings suggest that ChR2 stimulation of one hedonic hotspot recruits fos activity of the other hedonic hotspots in order to amplify hedonic 'liking'; expressions. Overall, our findings suggest the existence of novel hedonic hotspots in brain corticolimbic sites that form a functional hedonic circuit for brain 'liking'; control.

Positive allosteric modulation of alpha7 nicotinic acetylcholine receptors enhances sustained attention and decreases anxiety-like behavior after experimental traumatic brain injury

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Traumatic brain injury (TBI) is a leading cause of cognitive disability. Although long-term impairments in higher-order executive functioning like sustained attention are common clinically, they have not been fully evaluated in preclinical TBI models. TBI-associated psychopathologies such as anxiety can also further exacerbate cognition. TBI-induced cholinergic dysfunction is a mechanistic factor underlying sustained cognitive impairments and thus pharmacological strategies that enhance acetylcholine (ACh) receptor subtypes may ameliorate deficits. We hypothesized that NS-1738, a novel positive allosteric modulator at the Î±7 nicotinic ACh receptors, will restore sustained attention and reduce anxiety-like behavior after TBI. Anesthetized adult male rats received either a controlled cortical impact of moderate severity (2.8 mm tissue deformation at 4 m/s) or sham injury (i.e., no impact) and then were randomly assigned to NS-1738 (3 mg/kg) or vehicle (1.0 mL/kg). Treatments began 24 h post-surgery and were given once daily for 7 days. Assessment of sustained attention and impulsivity was assessed on post-operative days 14-24 using the 3-choice serial reaction time task (3-CSRT). Anxiety-like responses (open field test; OFT) and passive/active avoidance (shock probe defensive burying test; SPDB) were conducted on post-operative days 28 and 29, respectively. Markers of cholinergic neurotransmission in regions that mediate attentional tasks were assessed via Western blotting. Statistical analyses included repeated measures ANOVAs and when appropriate the Newman-Keuls post hoc test. The data suggest that NS-1738 ameliorates TBI-induced deficits in the 3-CSRT and OFT, and enhances measures of active coping in the SPDB, which supports the hypothesis and promotes a novel pharmacotherapy for attenuating cognitive deficits and affect disorder for TBI. Funding: NS110609, NS007433, Children’s Hospital of Pittsburgh

Sex differences in sodium butyrate enhancement of conditioned taste aversion.

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Animals can easily develop new food preferences and aversions through associative learning. If they consume a novel flavor and experience malaise, they develop an aversion towards that particular flavor, food or fluid. Conditioned taste aversion (CTA) requires gene expression and histone acetylation, which can be enhanced by HDAC inhibitors such as sodium butyrate (NaB). Here, we sought to determine whether enhancement of CTA using NaB is subject to sex-specific difference through changes in histone modification. Adult male (n=32) and female (n=32) Sprague-Dawley rats were placed on water restriction for 7 days with ad lib food to ensure intake on conditioning day. On the conditioning day, they were given 30 min access to saccharin (0.125%) and then injected with either sodium chloride (NaCl) or lithium chloride (LiCl; 0.15M, 3ml/kg). Ten minutes later, half the rats were injected with NaB (0.3M, 12ml/kg) or NaCl (0.3M, 12ml/kg. Thus, there were 4 groups for each sex: NaCl-NaCl, NaCl-NaB, LiCl-NaCl, and LiCl-NaB. Beginning the next day, 2-bottle 24-h preference tests of water vs. saccharin was carried out for 10 days. NaCl-NaCl and NaCl-NaB groups showed high preference for saccharin throughout 2-bottle testing. Male LiCl-NaCl showed moderate CTA which extinguished over the 10 days of preference testing. Male rats injected with LiCl-NaB appeared to have a greater CTA than LiCl-NaCl rats. However, NaB did not appear to enhance CTA in the LiCl-NaB-injected females compared to LiCl-NaCl-injected rats. Thus, female rats appear to be less sensitive to the CTA-potentiated effects of NaB. This could be due to (a) sex differences in pharmacokinetics or (b) sex differences in gene-regulation during CTA learning and its modulation by histone acetylation. Future studies could explore the role of estrogen on NaB effects, e.g., by testing the enhancement of CTA by NaB in ovariectomized female rats.Funding: Department of Biological Science

'Wanting what hurts': Central amygdala neuronal subtypes in the pursuit of reward and pain

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Incentive motivation that is dissociated from actual outcome value can lead to maladaptive attractions, such as in the case of a rat that pursues a painful shock-delivering object. We have previously shown that optogenetic channelrhodopsin (ChR2) stimulation of the central nucleus of the amygdala (CeA), when paired with the presence of a painful shock-rod, causes laboratory rats to repeatedly self-inflict shocks. However, the CeA is a heterogeneous structure and the contributions of its individual neuronal subpopulations in creating shock-rod attraction is not known. Here, in laboratory rats, CeA neurons expressing D1, adenosine 2a (A2a), or corticotropin releasing factor (CRF) receptors were optogenetically stimulated via ChR2 in the presence of a shock-rod. D1-stimulated rats displayed intense attraction for the shock-rod, repeatedly self-inflicting shocks and even overcoming an occluding barrier to do so. Shock-associated auditory cues also gained incentive value and were pursued in a separate operant task in the absence of optogenetic stimulation. In another separate self-stimulation operant task, optogenetic ChR2 stimulation on its own was also pursued. CRF-stimulated rats displayed only mild attraction for the shock-rod, and A2a-stimulated rats showed no attraction. These results reveal individual contributions of CeA neuronal subpopulations in producing maladaptive attraction, and may have implications for understanding the brain mechanisms of disorders in which individuals pursue unwanted and even harmful stimuli, such as in addictions, obsessive compulsion, and self-harm.This work was supported by National Institutes of Health grants MH063649, DA015188, and T32DA7268

Adolescent social isolation alters reward-seeking behavior in mice.

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Social isolation (SI) during adolescence is a risk factor for psychiatric illness in adulthood. Human studies of SI have shown a phenotype of impaired cognition and an increased risk of substance use disorders. Rodent models of SI have shown various behavioral and neurological abnormalities, but so far there has been no conclusive mechanism found linking SI to the observed phenotype. We previously reported findings that the transcription factor deltaFosB, a truncated isoform of FosB which accumulates following chronic stress, is increased following adolescent SI. Increases in deltaFosB have also been observed in response to chronic drug exposure, and have been linked to increases in reward-seeking behavior in rodents. The continuous performance task (CPT) is a touchscreen-based task that is sensitive to changes in sustained attention and vigilance, and is translatable between humans and rodents. Our adolescent social isolation rearing model consisted of isolation housing during postnatal days 21-35, followed by re-housing with another isolated littermate until postnatal day 63, at which behavioral tests were administered. We found that adolescent social isolation enhanced performance in the CPT in male SI mice. This was contradictory to our hypothesis that adolescent SI would be detrimental to attention, so we also tested mice in a fixed ratio/progressive ratio paradigm to test if reward-seeking behavior was driving the CPT results. We observed increased reward-seeking behavior in male SI mice. Sex-based differences in CPT performance provide valuable information about the manifestation of the SI phenotype. The CPT and progressive ratio results highlight an interesting interaction between the effects of SI on attention and its effects on reward-seeking behavior. The reward-seeking behavior changes in particular are notable because of the potential application to the susceptibility to substance use disorders seen in humans. This research was funded by the Lieber Institute for Brain Development.

Dopaminergic plasticity across contingency learning.

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Deficits in reward learning and motivation in diseases such as anxiety, depression and substance use disorder have been directly linked to experience-induced alterations in dopamine transmission in the ventral tegmental area (VTA) to nucleus accumbens (NAc) pathway. Previous work has aimed to define the molecular basis of the pathological expression of disease in the dopamine system to define targets to reverse or ameliorate disease symptoms. Moreover, a majority of work outlining the underlying mechanisms of dopaminergic plasticity has centered on electrophysiology at cell bodies or release mechanisms at terminals. However, dopamine is released from multiple cellular compartments â€“ including both somatodendritic and terminal compartments, regulated by different mechanisms - yet relatively little is understood about these mechanisms in non-pathological context. Using ex vivo fast-scan cyclic voltammetry (FSCV) and optical slice imaging methods paired with pharmacology, we characterized compartment-specific plasticity at various points across learning. We reveal sex-specific and compartment-specific experience-dependent plasticity in dopamine release mechanisms as an important neural substrate of reward learning. Our results indicate enhanced phasic stimulated release in male, but not female, animals following contingency learning, associated with distinct aspects of behavior during the task. Further, we utilized fiber photometry to record in vivo dopamine kinetics in both the terminal and somatodendritic compartments, revealing distinct activity signatures across contingency learning, suggestive of a regulatory role for somatodendritic dopamine release across the task. Overall, the results of these experiments support temporally- and compartment-specific roles of dopamine in basal cognitive functions, with broad implications regarding the critical nature of dopamine plasticity in specific learning events and ultimately further extend our understanding of these processes in both health and disease. Work was supported by NIH DA04211, DA048931, DA045103, DA051136; and the Brain and Behavior Research Foundation, the Brain Research Foundation, Alkermes Pharmaceuticals, Whitehall Foundation, the Edward Mallinckrodt Jr. Foundation.

Phenotypes of reinforcement sensitivity as predictors of the response to acute antidepressant treatment in rats

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One of the biggest threats to modern societies is the increasing prevalence of mood disorders. Cognitive deficits associated with depressive and bipolar disorders are a major driver of functional impairment and the ensuing disability of the suffering individuals. Growing evidence has indicated strong inter-individual differences in the vulnerability to development and effectivity of treatment of these psychiatric conditions, linking various levels of reinforcement sensitivity with specific mood conditions. In this study, we took a unique opportunity to investigate how trait sensitivity to reinforcement determines the reactivity of rats to acute antidepressant treatment. For this, using a preclinical version of the probabilistic reversal-learning (PRL) paradigm, we identified 4 phenotypes of sensitivity to negative and positive feedback in rats, which could represent various types of potential vulnerability to affective disorders. Subsequently, using the light/dark box (LDB) and progressive ratio schedule of reinforcement (PRSR) tests, we evaluated inter-phenotypic differences in the effects of acute treatment with 3 different antidepressant drugs (escitalopram, mirtazapine, and clomipramine, each in 3 doses) on anxiety and appetitive motivation of experimental animals. We report statistically significant differences between the investigated phenotypes of reinforcement sensitivity in the effects of acute escitalopram treatment on anxiety in the LDB test. We also report phenotype-independent effects of mirtazapine on motivation and anxiety and a lack of effect of clomipramine. These results demonstrate for the first time that trait sensitivity to reinforcement could have important implications for the effectiveness of treatment in affective disorders. Funding: this work was supported by the Polish National Science Centre (Research grant 2016/23/B/NZ4/01562 to RR) and by the statutory funds of the Maj Institute of Pharmacology Polish Academy of Sciences.

The Effect of Early Life Adversity on Risky Decision Making in Male and Female Rats.

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Early life trauma can increase the likelihood of developing future psychiatric disorders, such as substance use disorder, that are linked to risky decision making. However, few studies have examined whether mild early life adversity (ELA) alters risky choice in adulthood. Here we tested whether a brief exposure to a low resource environment early in life alters risky decisions in male and female rats in adulthood. To induce ELA, we implemented the limited bedding and nesting (LBN) model. In the LBN model, rat dams and their pups are placed in a cage with limited access to bedding and nesting materials from postnatal days 2-9. As adults, we measured whether LBN exposure affects their risky choice on the probability discounting (PD) task. In PD, rats are required to choose between a lever that always delivers a small reward (certain lever), and a second lever that occasionally delivers a large reward (risky lever). Animals train on one of two variations of the task: ascending vs descending. In the ascending version, probabilities of earning the large reward start low (6.25% in the first block) but increase throughout the task (100% in the final 5th block). The opposite happens in the descending version. We found LBN and control rats risky choice in either variation of the task were similar. However, LBN females showed a reduced tendency to choose the risky option after being rewarded for the risky choice on a preceding trial (i.e., win-stay). Thus, LBN may have a subtle effect in reducing risky choice in females. Our prior research found LBN reduced impulsive choice in males. Together, these studies could suggest that this mild form of ELA can reduce risky and impulsive behaviors in a sex-specific manner. These effects may be protective in a dangerous environment and reflect stress resilience.

Effects of genotype on temporal discounting in the TgF344AD rat model of Alzheimer's Disease

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The relative weight that individuals give to rewards and â€œcostsâ€• (such as delay to reward delivery) in making decisions varies significantly across populations and disease states. One aspect of decision making involves weighing benefits and costs associated with immediate and delayed outcomes. This aspect of decision making is referred to as temporal discounting and can be assessed on tasks in which subjects are required to choose between small, immediate rewards and larger rewards delivered after varying delays. Recent literature shows there is a great deal of variability in the temporal discounting phenotype between individuals with mild cognitive impairment, frontal temporal dementia, and Alzheimer's Disease (AD), with some showing no differences relative to healthy controls and others showing altered rates of temporal discounting. Beyond these disorders, individual differences in choice behavior (either maladaptive or normative) in young adults predict a variety of life outcomes, including educational success and socioeconomic status. The current study used a behavioral approach to determine the effect of genotype on phenotypic differences in temporal discounting in the TgF344AD (Tg) rat model of AD. Young adult rats non-Tg and Tg were trained on a temporal discounting task in which preference for small vs. large rewards was evaluated in presence of increasing delays to large rewards. Analyses on choice performance revealed Tg rats showed a greater preference for the large reward at the longer delays relative to non-Tg. This effect was not due to a difference in trial engagement as both groups showed an equal number of trials completed. Interestingly, young Tg rats showed a similar decision-making phenotype as old rats and these data suggest AD pathology may manifest as maladaptive decision making early in life.Support: Interdisciplinary Training in Pathobiology and Rehabilitation Medicine 2T32HD071866-06 to CMH and R01 AG066489 to LLM.

Intermittent-access self-administration model results in higher motivation for heroin in adult rats.

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Opioid abuse has been a rapidly growing threat to the health of Americans in the past decade, with overdose deaths and hospitalizations on the rise. While past studies have traditionally utilized continuous-access self-administration models to investigate drug-directed motivation, more recent researchers have switched to intermittent-access (IntA) self-administration as a novel animal model that better represents human patterns of drug-taking. Subjects receive five-minute drug-accessible periods separated by 25-minute drug-unavailable periods, instead of continuous long access (LgA) to the drug. Studies in nicotine and cocaine have successfully demonstrated increased drug-directed motivation using this paradigm while illustrating unique, â€œspikingâ€• patterns of drug levels in the brain, creating alternating periods of intoxication and sobriety. The current study's objective was to test this self-administration model's utility with opioids such as heroin. We used behavioral economics measures such as progressive ratio and threshold testing to evaluate motivation before and after the self-administration period. During self-administration, we found that IntA rats took less heroin than their LgA counterparts and female rats took more heroin than male rats. During progressive ratio, where responding requirements increased after each infusion, IntA rats significantly increased responding after self-administration while LgA rats' responding remained unchanged. Surprisingly, male rats responded more than female rats, which departs from previous studies regarding sex differences in drug-taking. Additionally, we found that IntA rats had a higher maximum behavioral â€œpriceâ€• they were willing to pay during threshold testing, where the dose amounts decreased as the session proceeded and more responding was required to receive a consistent reward. These results suggest that heroin may follow similar underlying mechanisms and behaviors as other drugs of abuse, opening up further opportunities to study therapeutic methods to combat compulsive drug-taking behaviors in opioid addiction.

Behavioral differences in adolescent rats during instrumental learning and attentional set-shifting: A comparison of two models of pediatric TBI.

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Traumatic brain injuries (TBIs) account for 800,000 emergency department admissions of children in the U.S. yearly. Survivors have a higher risk of repeated mild insults or concussions, known as rmTBIs, which can lead to delayed development as well as enhanced propensity for attentional and mood disorders. Preclinical models of attention after TBIs have not been cross-referenced across different injury types, leaving the possibility that injury modality differentially alters behavior. We aimed to compare behavioral assays (goal-directed behavior, motivation, and attention) in adolescent rats following rmTBIs [closed-head injury daily on postnatal days (PND) 17-19, 1 mm depth at 4 m/s] versus a one-time controlled cortical impact (CCI) (focal injury, PND 17, 2.2 mm depth at 4 m/s) as pediatrics. Motivation-based behavior was measured through an instrumental learning task (ILT) over 12 training days during adolescence, starting two weeks post injury. Results were analyzed using repeated-measures ANOVAs, followed by Newman-Keuls post hoc tests. The ILT revealed no significant behavioral changes between CCI and rmTBI groups. However, adolescent rats in both TBI groups completed more total trials, made fewer task-irrelevant pokes, had shorter cue-to-poke and poke-to-reward retrieval latencies compared to sham rats (p's<0.05), contrary to our hypothesis that TBI rats would underperform. The findings suggest divergent interpretations, such as enhanced motivation, yet reduced multi-tasking and exploratory behavior in adolescent rats after pediatric TBI. Behavioral flexibility was tested by a digging-based attentional set-shifting test (AST) with increasingly difficult contingencies for a food reward. No differences were found between CCI and rmTBI rats, or between TBI groups and Sham rats, likely due to a well-described cognitive rigidity exhibited by adolescents on AST. Further research using clinically-relevant injury modalities to predict post-injury behavioral symptoms in survivors of childhood TBIs is therefore warranted. Funding: NIH R21NS099683-01 and Childrens' Hospital of UPMC (PI: Bondi).

Inputs to Sex-Different Vasopressin Neurons in the Bed Nucleus of the Stria Terminalis

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The neuropeptide arginine-vasopressin (AVP) has long been implicated in the regulation of social behavior and communication. Ablations of the sex-different AVP-expressing cells within the bed nucleus of the stria terminalis (BNST) result in sex-different effects on these behaviors, however, the specific inputs to BNST AVP cells are completely unknown. Here, we use a cre-dependent, modified rabies virus (RV) tracing strategy in combination with AVP-iCre mice to map out the monosynaptic inputs to the BNST AVP cells. Male AVP-iCre mice received unilateral co-injections of adeno-associated viruses (AAVs) carrying Cre-dependent TVA-eGFP (for RV cell infection) and G (for transsynaptic retrograde infection) constructs into the BNST, followed by an injection of RV in the same region. Following a 7-day incubation, subjects were sacrificed and processed to identify BNST AVP neurons (mCherry + eGFP labeled; starter cells) and input cells (mCherry label only) throughout the brain. Preliminary results indicate that BNST AVP cells in male mice receive the strongest input from the lateral septum and extended amygdala structures (medial amygdaloid nucleus), lateral and medial preoptic areas, lateral, anterior, and ventromedial hypothalamic areas, and the paraventricular nucleus. More moderate input comes from basal forebrain (ventral diagonal band of Broca), ventral basal ganglia structures (nucleus accumbens shell, ventral pallidum), as well as from the septohypothalamic nucleus and basomedial amygdala. In addition, BNST AVP cells received minor inputs from olfactory structures (piriform cortex, olfactory tubercle), additional basal forebrain and ganglia regions (nucleus accumbens core, substantia innominata, horizontal diagonal band of Broca), amygdala (anterior, central, anterior cortical, posterior cortical and extended amygdala), medial septum, the premamillary and arcuate hypothalamic nuclei, epithalamic and thalamic areas (paraventricular thalamic nucleus, lateral habenular nucleus), and from midbrain (substantia nigra, dorsal raphe). No labeling was noticed in hindbrain regions. Overall, these results indicate that BNST AVP cells receive input from regions known to regulate social and motivated behaviors. Research reported in this poster was supported by R01 MH121603 to AP and GJD; and F31 MH125659 to NR.

Early pup removal leads to social dysfunction and dopamine deficit in late postpartum rats that is prevented by social support.

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Offspring interaction is among the most highly motivated behaviors in maternal mammals and is mediated by mesolimbic dopamine (DA) system activation. Disruption or loss of significant social relationships are among the strongest individual predictors of affective dysregulation and depression onset in humans. However, little is known regarding the effects of disrupted mother-infant attachment (pup removal) in rat dams. Here we tested the effects of permanent pup removal in rat dams, which were assigned to one of 3 groups on postpartum day (PD) 1: pups; pups removed, single-housed; or pups removed, co-housed with another dam who also had pups removed; and underwent a behavioral test battery during PD 21-23. In vivo electrophysiological recordings of ventral tegmental area (VTA) DA neurons were performed on PD22-23 in a subset of animals. Pup removal did not impact sucrose consumption or anxiety-like behavior, but increased passive forced swim test (FST) coping responses. Pup removal effects on social behavior and VTA activity were sensitive to social buffering: only single-housed dams exhibited reduced social motivation and decreased numbers of active DA neurons. Dams that had pups removed and were co-housed did not exhibit changes in social behavior or VTA function. Moreover, no changes in social behavior, FST coping or VTA activity were found in socially isolated adult virgin females, indicating that effects observed in dams are specific to pup loss. Thus, we show that deprivation of species-expected social relationships (pups) during the postpartum precipitates an enduring negative affect state (enhanced passive coping, blunted social motivation) and attenuated VTA DA function in the dam, and that a subset of these effects is partially ameliorated through social buffering.Funding Acknowledgements: Supported by the National Institute of Mental Health (NIMH) under award number R01-MH101180 to A.A.G.

A novel, open-source, high-throughput monitoring system for maternal behavior in mice.

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Understanding the neural basis of maternal behavior requires a comprehensive analysis of the repertoire that parents express, under conditions that enable high-resolution monitoring of most if not all behaviors 24 hours/day for weeks to months. To achieve this, we designed a semi-automated habitat for mice using open-source tools, where behavioral data can be collected and saved near-continuously over 4+ months. Our system is soundproofed, temperature- and humidity-controlled, and supports neural recordings and gain-of-function and loss-of-function interventions. Our overall goal is comprehensive behavioral phenotyping throughout life and over generations. To date we have monitored 10 wild type (WT) dams across 2-4 litters apiece, examining changes across different aspects of maternal care from litter to litter. While infanticide is quite common in first-time mothers, the incidence decreases in most dams with the second and subsequent litters. We found that even before birth of the first litter, quality of nest-making predicted degree and perseverance of infanticide. Those dams that continued to be infanticidal stopped this behavior when co-housed with experienced dams that were not infanticidal. Previously we found that the neuropeptide oxytocin promotes long-term plasticity and mediates experience-dependent changes in neural circuits that increase the sensitivity to pup cues, thereby facilitating behavioral responses to pup distress. To understand the effects of genetic disruption of oxytocin signaling in maternal care, we monitored the first pregnancies of 2 oxytocin receptor knockout (OXTR KO) dams. These OXTR KO animals built subpar nests, spent less time interacting with their pups, and had high pup mortality rates compared to WT mice who raised successful first litters. Interestingly, WT dams who neglected or cannibalized their pups showed similar nest-building patterns and time spent in the nest as the OXTR KO mice. This work is supported by the NIH (1U19NS107616-01) and the NSF (1650114).

Investigating neural circuit mechanisms that govern scratching frequency

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Itch is an unpleasant sensory experience which elicits bouts of scratching which can vary in length of time. However, the neurobiological basis governing how long we itch is unknown. Defects in this system can cause pathologies such as chronic itch in which bouts of scratching are prolonged. Therefore, understanding how the nervous system encodes scratching duration is of great clinical relevance. One of the main limitations in previous rodent models is that pharmacological agents used to induce itch (such as chloroquine and histamine) have variable lengths of action and diffusion kinetics through a tissue. Here we circumvent these limitations and optogenetically activate of a defined itch-specific cell populationâ€"MrgprA3+ sensory neuronsâ€"and examine how itch frequency is affected in vivo. Using a transgenic mouse line in which MrgprA3-Cre drives ChR2 only in A3 neurons, we activated these itch-specific cells in both the skin of the cheek and the nape of the neck for 5 minutes each. We found that the number of scratching bouts was dramatically higher in the neck as compared to the cheek, using an identical transdermal optogenetic stimulus. In order to further probe the neurophysiological basis of this difference, we then reasoned that there may be differential gene expression in the A3 populations which innervate the cheek and skin, respectively. The cheek skin is innervated by the trigeminal ganglia (TG) and the neck is innervated by dorsal root ganglia (DRG), so we performed RNA sequencing of A3 neurons from TG and DRG. This sequencing data showed that A3 neurons in the TG express much higher levels of a pain-inducing peptide, prolactin. Pain is known to the capable of suppressing itch, so one possibility is that higher prolactin signaling in A3+ TG neurons leads to more pain than in DRG, which in turn is responsible for suppressing itch in the cheek. Taken as a whole, these results not only provide us with a new mouse model for studying itch frequency at the behavioral level, but also present an exciting opportunity to dissect the neurobiology of scratching frequency in greater detail.

Oral cannabidiol administration: Assessing bioavailability and behavioral outcomes in a rodent model

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There has been a recent surge in popularity for cannabidiol (CBD), a major non-psychotropic constituent of cannabis, due to numerous claims of potential therapeutic properties, which include, but are not limited to, anxiolytic, antinociceptive, and anti-inflammatory effects. However, as previous scientific literature on CBD's effectiveness in providing such therapeutic effects is limited, this project was aimed to evaluate the potential beneficial properties of a hemp derived 99% pure CBD compound provided from Ellipse Analytics (Denver, CO) in a rodent model. We analyzed the pharmacokinetics of this CBD product as well as the behavioral outcomes after acute and chronic administration. Pharmacokinetics of CBD were assessed by administering CBD to adult male rats (n=10/group) using oral gavage at 0, 5, 10, 20 or 40 mg/kg in sesame oil for 10 days while drawing blood 1hr, 2hr, and 4hrs post administration on the 1st, 5th and 10th day to measure plasma CBD levels. In a second and third cohort of rats (n=16/group), CBD was administered by oral gavage at a 0, 20, or 40 mg/kg in sesame oil over 10 days, with behavioral assays being run on the 1st, 5th, and 10th day to assess outcomes associated with pain response, sleep behavior, anxiety response, and stress levels. Our results showed a dose dependent increase in CBD bioavailability with plasma levels peaking between the 1st and 2nd hour post-administration, and significantly decreasing by the 4th hour across all groups. There was a minimal effect of CBD on sleep, pain, and anxiety-like outcomes, however 40 mg/kg CBD significantly decreased corticosterone levels during restraint stress as compared to controls. These findings provide evidence for a potential therapeutic effect of CBD on hypothalamic pituitary axis function and thus stress regulation. Further analysis is necessary to assess the potential for dose dependent increases in overall therapeutic effectiveness with acute or prolonged exposures in males and females. funding provided by Ellipse Analytics (Denver, Colorado)

Dorsal Peduncular Prefrontal Cortex contains cells uniquely sensitive to opioids: Relevance to opioid reward and addiction.

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The US is in the midst of an opioid abuse and overdose epidemic, with over 200 people dying each day from opioid overdose. The vast majority of research on opioid addiction has focused on a small number of circuits, primarily the mesocorticolimbic circuit. We used iDISCO+ tissue clearing and c-Fos staining to create a whole-brain map of transcriptionally responsive neurons following acute oxycodone (5 mg/kg) or saline injection. This revealed 39 regions with significant induction of c-Fos expression following oxycodone. We chose to further examine the dorsal peduncular area (DP), the ventral-most component of the medial prefrontal cortex (mPFC). Single-cell sequencing, qPCR, and RNAscope revealed unique properties of opioid-responsive DP neurons relative to surrounding mPFC. The DP is enriched in Oprm1 (Âµ opioid receptor) and Slc17a6 (vGlut2) relative to neighboring mPFC. Surprisingly, Oprm1 and Slc17a6 are co-expressed in DP neurons in layer 5 that show robust transcriptional responses to oxycodone. Patch-clamp recording showed that Oprm1-expressing neurons have relatively depolarized resting membrane potential, and larger Ih currents than nearby Oprm1(-) neurons. Using FosTRAP mice, we â€˜tagged' opioid-responsive neurons DP, and optogenetic stimulation of this ensemble produced aversion-related behaviors that were blocked by oxycodone. Further, optical stimulation of DP in opioid-dependent mice enhanced naloxone-precipitated withdrawal symptoms. Selectively deleting Oprm1 in the DP of Oprm1-floxed mice reversed the hedonic valence of oxycodone, and reduced oxycodone self-administration. Whole-brain projection mapping revealed opioid-responsive DP neurons project to several mid- and hindbrain sites with known involvement in aversive behaviors, including the parabrachial nucleus (PBN). Optical stimulation of DP-PBN circuit recapitulated the aversive phenotype seen during DP somatic stimulation. Thus, the DP is a novel prefrontal site that regulates opioid reward and dependence. Funding: NARSAD Young Investigator Award (ACWS), NIDA K99 (ACWS), NIDA R01 (PJK).

Neuronal ensembles within the prelimbic cortex control recently acquired cocaine-seeking behavior in rats.

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Neuronal ensembles within the Infralimbic cortex (IL) rapidly develop after behavioral acquisition for food seeking. Furthermore, ensembles within the IL mediate both food seeking and cocaine seeking in well-trained rats. It remains unclear whether neuronal ensembles are gradually formed and refined over the course of extensive cocaine self-administration, or if functionally relevant ensembles might be recruited and formed as early as the initial acquisition of cocaine self-administration behavior. Here we test the hypothesis that neuronal ensembles within the Prelimbic cortext (PrL) form rapidly after rats acquire lever-pressing behavior for infusions of cocaine (0.75mg/kg/infusion). To test this hypothesis, we developed a novel behavioral procedure to probe the role of neuronal ensembles following initial acquisition of lever pressing behavior for cocaine infusions. We trained adult male and female Fos-LacZ rats to self-administer cocaine between 1 - 12 days, until rats meet criteria for acquisition of cocaine seeking (â‰¥30 active lever presses and â‰¥70% total responding on the active lever). Twenty-four hours later, we put rats through a 30-minute non-reinforced seeking test to induce Fos and Î²-galactosidase co-expression. Ninety minutes later, we infused Daun02 into the PrL. We reasoned that Daun02 infusions would interact with Î²-galactosidase, resulting in selective ablation of neurons activated during the cocaine-seeking test and disrupt recall of the recently acquired cocaine-seeking behavior. We found that Daun02 significantly decreased active lever pressing for cocaine compared to vehicle (p<.05). This finding indicates that neuronal ensembles within the PrL are formed as early as one day after learning and mediate cocaine seeking. Acknowledgements: NIDA DA042102, BBRF:Young Investigator Award, UF COP.

Brain-wide identification of the neural ensembles underlying fear generalization in mice.

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A major feature of post-traumatic stress disorder (PTSD) is fear generalization, which occurs when a fearful response of a past experience generalizes to novel situations. Fear generalization can lead to heightened anxiety and maladaptive responses in safe environments. The representation of a fear memory is thought to be encoded in the fear memory trace or engram, which is the neural ensemble active during fear encoding whose reactivation by the original stimulus results in memory retrieval. It is not known if the fear engram is inappropriately reactivated in safe contexts during fear generalization. Here, we addressed this gap in knowledge by investigating how fear engrams are activated during high and low fear generalization. We used a contextual fear discrimination (CFD) paradigm in which we deliver a shock to a mouse in context A and then measure if they can discriminate between this aversive context and a similar, but safe context B. To visualize engrams, we utilized our activity-dependent tagging system, the ArcCreERT2 mice. This system allows for the permanent labeling of neurons active during fear encoding, which can then be compared with the ensembles activated during retrieval. First, mice were administered a contextual fear conditioning protocol in context A and their fear-encoding neural ensembles were tagged. Five days later, mice were administered the CFD protocol each day. We show that fear generalization decreases with repeated context exposures. Mice were euthanized on Day 3 or 10 of CFD (high and low fear generalization, respectively). Our data shows that a lower percentage of cells in the dentate gyrus (DG) is reactivated during memory retrieval in the low fear generalization group compared to the high fear generalization group. Our results suggest that the DG fear engram becomes more specific after fear discrimination learning. We are currently examining the neural ensembles corresponding to high and low fear generalization in various fear-processing brain areas. Our findings will enhance our understanding of how fear is represented in the brain and may provide us with novel targets for the treatment of fear disorders such as PTSD. Funding: Whitehall Foundation Grant, NIH Transformative Award.

Different exercise tendencies modulate behavioral and molecular changes to opioid or exercise-induced reward.

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Reward changes were observed in rodents with different exercise tendencies by utilizing the conditioned place preference (CPP) paradigm. Colleagues at the University of Missouri - Columbia established three distinct genotypes with reliable phenotypes among Wistar rats: low volume runners (LVR), high volume runners (HVR), and wild-types (WT). Adult male Wistar rats from each of these groups were given a rewarding stimulus of either access to a running wheel or an injection of morphine. Results indicated that there was no difference in the strength of CPP between the rewarding stimuli. Extinction was significantly more effective in LVR than HVR and WT animals, as LVR animals spent a lower percentage of time in their assigned conditioning chambers after eight days of exposure to the CPP paradigm without a rewarding stimulus. It was confirmed that running wheel access and opioid administration were comparably rewarding in all groups. The conclusion is that, compared to HVR and WT, LVR animals with their different genotypes and phenotype also have a unique pattern of behavioral responses to an opioid and to running.

Maternal immune activation modifies ventral hippocampal regulation of social stress

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Environmental enrichment (EE) has been successfully implemented in human rehabilitation settings, including demonstrated benefits for children with autism. However, the mechanisms underlying its success are not understood. Incorporating components of EE protocols into our animal models allows for the exploration of these mechanisms and their role in mitigation. Using a mouse model of MIA, the present study explored changes in social behavior and associated hypothalamic pituitary adrenal (HPA) axis functioning, and whether a supportive environment could prevent these effects. We show that prenatal immune activation of toll-like receptor 3, by the viral mimetic polyriboinosinic-polyribocytidilic acid (poly (I:C)), led to disrupted maternal care in that mouse dams built nests of poorer quality, compared to saline-treated controls (n = 8-14 litters per group). This MIA associated effect on maternal care was corrected by EE housing. Compared to saline treated mice, standard housed adult male and female poly (I:C) offspring engaged in higher rates of repetitive rearing and had lower levels of social interaction but did not demonstrate increased anxiety-like behavior or distance traveled in the open field test. Moreover, these animals had delayed recovery of plasma corticosterone and elevated ventral hippocampal expression of corticotropin releasing hormone (Crh) and Crh receptor 1 (Crhr1), in addition to glucocorticoid (Nr3c1), oxytocin receptor (Oxytr), protein kinase C alpha (Pkrca), and calcium/calmodulin dependent protein kinase II alpha (Camk2a) mRNA in response to an acute social stressor. Enrichment housing, likely mediated by improved maternal care, protected against these MIA-induced effects. These data demonstrate that augmentation of the environment can offset effects of early health adversity by buffering HPA dysregulation.

The contribution of the dorsal hippocampus to rat behavior in a changing aversive-appetitive task

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The hippocampus is important for episodic memory and spatial navigation. However, the rodent hippocampus is an elongated structure with multiple sub-regions. Most research has focused on the dorsal part of the hippocampus, closest to the brain surface, with less known regarding more ventral parts. The dorsal hippocampus is involved in spatial memory and navigation, while the ventral hippocampus seems more involved in stress and emotional memory. The current study examines the relationship between the dorsal and ventral regions using a task that combines both spatial and emotional components. We are quantifying behavior, electrophysiology and the effects of dorsal hippocampus inactivation. Rats alternate between two feeders on a horseshoe/U shaped maze. On some trials a tone cues the animal that a mild current is running through the floor at the tip of the maze. Rat behavior changed during safe and unsafe trials. On unsafe trials, animals showed an increased hesitation especially close to the shock zone. In parallel to the behavior, a microelectrode array will record activity from single units in the ventral hippocampus. Hippocampal principle neurons have been called place cells, showing spatial tuning as animals traverse an environment. Less is known regarding place cells in the ventral hippocampus compared to the dorsal hippocampus, especially with respect to an emotional event. Furthermore, strong connectivity between the dorsal and ventral hippocampus subregions has also been shown, but few studies have examined the physiological interaction between these two subregions. To determine the contribution of the dorsal hippocampus to the behavior and ventral place cell activity patterns, chemogenetics (DREADDS) will be used to transiently inactivate the dorsal hippocampus. This study will further our understanding of hippocampal function in a changing context and on the interplay along the longitudinal axis of the hippocampus. These results could also have important implications for psychiatric disorders involving fear and anxiety.

The multi-generational impact of exposure to opioids.

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Opioid use and abuse is devastating communities around the world. Often, initial opiate exposure occurs during adolescence, which represents a critical developmental window. Drug use during adolescence may affect future offspring, even when discontinued prior to conception. This talk will discuss results obtained utilizing a rodent model of adolescent opioid exposure in male or female rats. The offspring were tested for addiction-like behaviors utilizing opioids or cocaine in adulthood. The data indicate that offspring from rats adolescently exposed to morphine have altered drug intake patterns compared to those exposed to saline, which are sex-specific, drug-specific, and sex of exposed parent-specific. Molecular changes are also present within reward circuitry of adult F1 animals. Preliminary data will be discussed that examined potential mechanisms of transfer, including epigenetic changes in F0 testes and ovaries, and molecular changes across development in F1 offspring. This work was funded by NIHR03 DA034886.

Can CBD protect against toxic synergism of lipopolysaccharide and chronic restraint stress in female mice?

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Lippopolysaccride (LPS) is a neuroinflammatory model for Parkinson's disease (PD), where dopamine neurons (DA) in the substantia nigra (SN) die. Single High doses of LPS have been shown to induce chronic inflammation and DA death after a 7-month delay. Female mice have been shown to require multiple injections of LPS at high doses to elicit neurotoxic effects. However, because LPS and chronic restraint stress (CRS) have each been shown to increase oxidative stress and use similar mechanisms to induce reactive microglia. Thus, we investigated if repeated exposure to lower dose LPS, when paired with CRS may act synergistically to increase toxic effects earlier than the reported 7 months. Importantly, there are sex differences in reactivity of microglia, yet no research has examined chronic, and/or synergistic, effects of LPS and CRS in females. Cannabidiol (CBD) has been suggested to downregulate pro-inflammatory genes in microglia and may also act to increase resiliency via trophic factors. To investigate synergistic effects of two stressors, and protective effects of CBD, C57/Bl6J female mice were pair housed in standard housing and assigned to: Saline, Saline+ CRS, Saline +LPS, Saline +LPS+ CRS, CBD, CBD+ CRS, CBD +LPS, or CBD +LPS+ CRS groups. LPS groups received 1 mg/kg LPS interperitoneally once a week for 3 weeks. CRS groups received randomized restraint stress for 2 hours daily for 3 weeks. CBD groups simultaneously received 10 mg/kg of CBD i.p. twice a day at 12-hour intervals for 3 weeks. Brains were collected 3 months after the last injection. To assess vulnerability to PD, Tyrosine Hydroxylase (TH) positive DA neurons, and pro-inflammatory microglia indicated by co-labeling of Iba1 and Cox-2, were quantified in the SNpc. Surprisingly, there were no main effects of stressors ($p=0.894$), CBD ($p=0.510$) or an interaction ($p=0.244$) on the reactivity of microglia, microglia abundance, or TH in the SNpc. Suggesting that the toxic effects may be more chronic in nature, occurring after the 3-month timepoint used in this research. Further analysis will investigate the resiliency factors brain derived neurotropic factor and neuropeptide Y in the hippocampus and SNpc, to see if these mechanisms contribute to our observed neuroprotection.Funded by Schapiro Undergraduate Research Fellowship Award, RMC

Adolescent nicotine enhances morphine reward and produces paradoxical excitability of ventral tegmental area GABA neurons in adult mice.

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Opioid abuse is a major public health crisis. An important but poorly understood risk factor promoting opioid abuse is prior nicotine use. Epidemiological evidence suggests that adolescent nicotine acts as a gateway drug and increases one's vulnerability to subsequent drug abuse, including to morphine. However, despite these clinical findings, the neural mechanisms underlying this interaction remain unknown. Previous work has reported that the effects of adult nicotine exposure on ventral tegmental area (VTA) circuitry and on drug reinforcement behavior are prolonged if nicotine is administered during adolescence. This indicates that adolescence is a vulnerable developmental stage during which nicotine-related plasticity may be maintained into adulthood and thereby exert long-lasting influence over later-life drug use. To test the effect of adolescent nicotine on adult morphine reward, adolescent mice were exposed to nicotine in their drinking water for two weeks (0.1 mg/mL Days 1-5; 0.2 mg/mL Days 6-14) and behavioral- and circuit-level adaptations to morphine were assessed in adulthood. Adult mice which received adolescent nicotine exhibited heightened preference for the morphine-paired chamber during conditioned place preference relative to controls (water only). In addition, adolescent nicotine increased adult morphine locomotor sensitization, suggesting adolescent nicotine promotes morphine reward by enhancing drug sensitivity. These changes in behavior corresponded with alterations in VTA GABA signaling. VTA GABA neurons from adult mice exposed to adolescent nicotine demonstrated a depolarizing shift in the GABAA reversal potential and paradoxical heightened action potential firing in response to morphine. Together these data indicate adolescent nicotine promoted morphine reward in adult mice, at least in part by enhancing drug sensitivity, and that this is associated with increased excitation in VTA GABA neurons. Our results suggest that targeted pharmacotherapies that prevent or reverse nicotine-induced changes in VTA GABA signaling may provide an interventional approach to mitigate opioid abuse later in life. Work funded by NIH Grant R01-DA009411-19 (JAD).

Acute effects of cannabis on cognition in aging.

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In recent years there has been an increase in legalization of both recreational and medical cannabis across the United States, with individuals over the age of 65 being one of the fastest growing demographics of cannabis users. Across species, aged individuals exhibit deficits in cognitive functions supported by the prefrontal cortex (PFC) and the hippocampus. These same cognitive functions are impaired by acute administration of cannabis or delta-9-tetrahydrocannabinol (THC) in young subjects; however, effects in aged subjects have been less well evaluated. The primary goal of the current study was to use a rat model to determine whether the effects on cognition of acute exposure to cannabis smoke differ between young and aged subjects. Male and Female, fully mature young adult (6 months) and aged (24 months) Fischer 344 x Brown Norway F1 hybrid rats were tested on both a PFC-dependent delayed response working memory task and a hippocampal-dependent trial-unique non-match to location (TUNL) task in touchscreen operant chambers. The delayed response task required rats to remember the location of a visual stimulus over variable delay periods ranging from 0-24 s. The TUNL task required rats to remember the location of a visual stimulus with varying degrees of discriminability from other, distractor stimuli. A semi-randomized, within-subjects experimental design was used such that each rat was exposed to smoke from burning, 0, 3, and 5 cigarettes immediately prior to test sessions in each task. In the delayed response task, acute exposure to cannabis smoke impaired accuracy in young rats but enhanced accuracy in aged rats. In contrast, in the TUNL task, cannabis smoke had no effects on performance in either age group. Considered together, this pattern of results suggests that in aged rats, which exhibit impaired cognitive performance, cannabis smoke can enhance PFC-dependent cognition, but has no effect on hippocampus-dependent cognition.

Ultrasonic vocalization in the context of social interaction and social preference in dopamine transporter knockout mice.

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Ultrasonic vocalizations (USV) are emitted by rodents as a form of communication. USV is most commonly emitted during circumstances that are emotionally evocative and reflects aspects of affective states. This includes a number of social circumstances, although the precise relationships between USV and social behavior have not been established. Examination of the types of USV emitted during social interaction will provide insight into the communicative properties of USV. Dopamine has a role in social play and social motivation. The dopamine transporter (DAT) regulates extra-synaptic dopamine levels. Elimination of DAT in heterozygous knockout (DAT +/-) mice thus provides a way to examine the effect of enhanced dopamine neurotransmission on social interaction and social preference. In the experiments, a 24-hr period of social isolation was used to increase social play. Methods: Male and female, DAT +/+ and DAT +/- mice were isolated for a 24-hr period, between 28 and 35 days of age, or remained in social housing. After this time, isolated mice were introduced to a novel cage for 10 min before a novel, socially housed mouse was introduced to the cage. Social and non-social behaviors were scored by an observer and USV was recorded. Results: Increased USV was associated with social behavior. Female mice emitted more USV compared to males. The longer the social interaction between the mice, the more complex the call types became. It appears that most calls were emitted when the mice were in close proximity of each other, in particular, during nose-to-anus and nose-to-nose sniffing. Conclusion: Social isolation affects the USV emission rate and quality, most evidently seen in females compared to male mice. Genotypic differences between DAT +/- and DAT +/+ mice suggest that extracellular levels of dopamine also influence the quality of USV emission, likely reflecting changes in social motivation. Understanding the role of dopamine in social motivation will provide insight into ASD, in which the primary deficits are of social motivation and social behavior. Funding Acknowledgement: Office of Undergraduate Research, University of Toledo.

Outcome-selective reinstatement involving choice is partially context-dependent, and is associated with activation of the dorsomedial striatum.

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A significant amount of research from both human and animal-based studies suggests that relapse to compulsive action is often provoked by exposure to certain contexts, but the vast majority of these data have been derived from situations involving a single active response. The context-specificity of relapse in situations involving choice between multiple actions and outcomes are less well-understood. We thus investigated how reinstatement involving choice between multiple actions was affected by altering the physical environment, or context. In Experiment 1, rats were trained to press a left lever for one food outcome (pellets or sucrose) and a right lever for the other food outcome, counterbalanced. Then, rats received a 30 min extinction session in either the same Context (A) or a Context (B). Rats were then immediately tested in either context A or B, yielding four groups overall: AAA, AAB, ABB, and ABA. Outcome-selective reinstatement is evident if rats respond on the same lever that earned the outcome during training. This result was observed for rats extinguished and tested in the same context (AAA and ABB), but non-specific reinstatement (Reinstated = Nonreinstated) was observed in groups that received extinction and testing in different contexts (AAB and ABA). Experiment 2 was conducted identically, except that rats received two 30 min sessions of extinction on separate days and were tested one day later. This time, all groups demonstrated intact outcome-selective reinstatement (Reinstated > Nonreinstated) regardless of context. Rats were perfused 2 hr after the start of test, and sections from the medial and lateral orbitofrontal cortices (mOFC and lOFC), dorsomedial striatum (DMS), and dorsal hippocampus (DH) were collected and immunostained for the immediate early gene and activation marker c-fos. Of these regions, only c-fos expression in the pDMS appeared related to the specificity of reinstatement performance. Overall, these results suggest that a) the specificity of outcome-selective reinstatement is transiently context dependent, immediately after extinction learning, and b) outcome-selective reinstatement performance is associated with neural activity in the pDMS. This work was supported by the ARC (DP200102445) awarded to L.A.B.

Excessive self-grooming behavior of BTBR T+Itpr3tf/J mice may serve a social signaling function.

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Research Autism spectrum disorder (ASD) is defined by two core behavioral characteristics; namely, restricted repetitive behaviors and impaired social-communicative functioning. BTBR T+Itpr3tf/J (BTBR) mice provide a valuable animal model for ASD to elucidate the underlying mechanisms of these two behavioral characteristics of ASD. This study examined the social function of excessive grooming behavior in BTBR mice as a phenotype of restricted repetitive behaviors. Compared to the control C57BL/6J (B6) strain, BTBR mice showed increased self-grooming when solely placed in a test apparatus, while it was more evident when confronted with a stimulus mouse (either B6 or BTBR) in the three-chamber test apparatus. While B6 mice tended to groom its face/snout region on the side of empty chamber, BTBR mice showed excessive grooming with frequent transitions among grooming body spots on the side of the chamber containing a social stimulus. Acute systemic injection of buspirone as an anxiolytic, facilitated approach behavior towards social stimuli in the three-chamber setting in both B6 and BTBR mice. However, this treatment did not affect grooming behavior in B6 mice and significantly enhanced self-grooming in BTBR mice. These behaviors of BTBR mice implicated a social signaling function of grooming in response to social stimuli, in which bodywide grooming of BTBR mice expressed in the proximity of social opponents may stimulate the release of olfactory (possibly dismissive) signals. Consequently, the putative neural mechanism underlying excessive grooming may differ from those regulating social approach that is associated with anxiolytic mechanism. Funding Acknowledgement This study was supported by MEXT KAKENHI Grant Number 19K24681.

Androgens-Vasopressin interplay: a key interaction for social recognition and aggression in male mice.

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Social recognition (SR) is a pivotal skill for social life in gregarious species. Several studies highlighted a role of vasopressin (AVP) in regulating aggressive and sociality. The AVP system includes sexually differentiated brain regions, such as the bed nucleus of the stria terminalis (BNST) and the lateral septum (LS). Androgens are strongly involved in the organization and activation of the AVP circuit. Moreover, social interactions rapidly change brain sex hormones' concentration, regulating behavior and suggesting that the AVP/androgen interplay impacts behavior with mechanisms currently poorly understood. To elucidate the rapid, non-genomic, effects of testosterone (T) on AVP neurons in the BNST, adult castrated male mice were intracerebrally infused with one of 4 different doses of T (0.25, 0.5, 0.75, 1ug of T in 0.5ul of 10% DMSO/side) targeting the BNST. They were then exposed to a difficult SR paradigm, in which castrated mice showed an impairment, and to a resident-intruder (RI) paradigm to assess aggression. In the difficult SR paradigm, mice were exposed to a choice between a novel and a familiar male mouse after 2 5-minutes sample session with 2 familiar males. For the RI paradigm, a different castrated male mouse (intruder) was introduced in the home cage of the experimental mouse for 5 minutes either 35- or 120-min post-infusion to evaluate rapid and long-lasting effects of T. To determine whether the effects of T were due to rapid conversion into 17beta-estradiol (E2) or dyhidrotestosterone (DHT) , the same behavioral tests were repeated in a different batch of animals receiving one of 3 different doses of E (25, 50 and 100nM of E in aCSF/side) or of 4 doses of DHT (0.0625, 0.125, 0.25, 0.5ug of T in 0.5ul of 10% DMSO/side). Results revealed that infusing T, E, or DHT facilitates SR, with male mice spending more time investigating a novel over a familiar castrated mouse, with an inverted U-shaped dose-response. In addition, T infusions did not elicit overt aggression towards the intruders, but mice receiving the highest doses of T showed a higher dominance score with long-lasting effects after 120 min. Intriguingly, also E and DHT increased the dominance score at 35 min and 120 min too. Elucidating the AVP-sex hormones interaction could lead to new therapeutic approaches for psychopathologies of social behavior with strong sex difference as autism spectrum disorder. This study was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

Impact of motor stroke on action metaphor comprehension

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Previous studies indicate that damage to motor regions negatively impacts literal action verb comprehension. However, whether or not damage to motor regions impacts action-related metaphors has not been tested. If metaphorical language is also impacted, it would support the notion that metaphors are grounded in sensorimotor representations. Here we test this hypothesis by assessing novel, conventional, and frozen metaphor comprehension in 14 right-handed adults with right-sided mild to moderate paresis following left hemisphere motor stroke and 24 control participants, all of whom had English as their first language. Novel metaphors consisted of newly created metaphors (e.g., â€œshe was skiing along an icy slopeâ€•), conventional metaphors consisted of familiar metaphors (e.g., â€œthey ran into each otherâ€•); and frozen metaphors consisted of idioms (e.g., â€œshe kicked the bucketâ€•). Metaphors were either action-related or non-action related. Consistent with our hypotheses, results indicated a significant group by metaphor-type interaction ($F(1, 35) = 4.385$, $p = .044$), such that only in the stroke group, accuracy with action-related metaphors were significantly lower than control metaphors (p=.002). Further, we found that novel action metaphors, compared to frozen action metaphors, were the most impacted by motor stroke (all ps<.05). These results strongly support the notion that motor-related brain regions are important not only for literal action comprehension, but also for action-related metaphors, especially when metaphors are novel.

The role of estrogens in dorsal hippocampal D2-type dopamine receptor mediated social learning in male and female mice.

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Social learning is a critical and adaptive form of learning that can be defined as learning that occurs via social interaction and/or social observation (Galef, 1988). The underlying neurobiological mechanisms that regulate social learning are poorly understood, but in animals may be tested using the social transmission of food preference (STFP) paradigm. During the STFP, dorsal hippocampus (HPC) infusions of the D2-type dopamine (DA) receptor antagonist raclopride blocked social learning in female but not male mice, suggesting an interaction with sex hormones (Matta et al., 2017). Later, we found that intra-HPC infusions of the same drug blocked social learning in gonadectomized female and male mice but not gonadally intact male or female mice (Bass et al., 2019). These findings suggest an interaction between D2-type DA receptors in the dorsal HPC and gonadal hormones in the regulation of social learning in mice. What is still unknown are the specific gonadal hormone (s) that may be involved. This study will begin by investigating the role of estradiol benzoate (EB) in D2-type DA facilitated social learning in mice and subsequent studies will investigate other hormones. Here, we implanted subcutaneous slow releasing silastic capsules of either EB or sesame oil in castrated (CAS) â€œobserverâ€• (OBS) mice in study 1 and in ovariectomized (OVX) OBS mice in study 2. During the experiment, OBSs received bilateral intra-HPC infusions of raclopride or a saline control 10-minutes prior to a 30-minute social interaction with a recently fed, same sex, gonadectomized â€œdemonstratorâ€• (DEM). OBSs then underwent an 8-hour choice test with free access to two novel flavored food diets, one of which their respective DEMs consumed before their social interaction. If social learning occurs, OBSs will prefer the DEM diet. Preliminary findings from study 1 revealed that long term EB treatment protects against the impairing effects of intra-HPC raclopride on social learning in CAS mice. These findings appear to confirm the hypothesis that dorsal HPC D2-type DA receptors interact with estrogen receptors to regulate social learning in male mice. It is predicted that EB will also protect against the block in social learning in OVX mice. Funded by NSERC.

Social, cognitive, and neurobiological outcomes in the Ube3a deletion rat model of Angelman Syndrome

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Angelman Syndrome (AS) is a rare neurodevelopmental disorder caused by dysfunction of neuronal ubiquitin protein ligase E3A (UBE3A) due to deficient maternal allele activity. AS is characterized by intellectual disabilities, impaired communication, motor deficits, microcephaly, and a happy, excitable demeanor with easily provoked laughter. To help address the unmet need of an effective therapeutic for AS, which persists despite decades of promising mouse research, a rat model of AS lacking maternal Ube3a was recently generated. We sought to delineate behavioral phenotypes in this model for future use as preclinical outcome measures and to investigate underlying neurobiology. We found typical responses to conspecific vocalizations and rates of vocalization during social interaction, but discovered juvenile and adulthood cognitive impairments and identified deficient hippocampal long-term potentiation as a putative underlying cellular mechanism. Structural neuroimaging illustrated profound reductions in brain volume that became more apparent with age. Furthermore, the severity of the neuroanatomical phenotype was correlated with pup ultrasonic vocalizations, which are reduced in this model. Taken together, our results indicate that the Ube3a maternal deletion rat offers a sophisticated rodent model of AS with high face validity to the human phenotype and provides robust behavioral and neurobiological outcome measures detectable at various life stages, which will be highly valuable in the ongoing pursuit of therapies for AS. This work was supported by the Foundation for Angelman Syndrome Therapeutics.

Pharmacological evidence of a cholinergic contribution to elevated impulsivity and risky decision making caused by adding win-paired cues to a rat gambling task

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Pairing rewards with sensory stimulation, in the form of auditory and visual cues, increases risky decision-making in both rats and humans. Understanding the neurobiological basis of this effect could help explain why electronic gambling machines are so addictive, and inform treatment development for compulsive gambling and gaming. Numerous studies implicate the dopamine system in mediating the motivational influence of reward-paired cues; recent data suggest the cholinergic system also plays a critical role. Previous work also indicates that cholinergic drugs alter decision-making under uncertainty. Aims: We investigated whether the addition of reward-concurrent cues to the rat gambling task (crGT) altered the effects of peripherally-administered cholinergic compounds. Methods: Muscarinic and nicotinic agonists and antagonists were administered to 16 male, Long Evans rats trained on the crGT. Measures of optimal/risky decision-making and motor impulsivity were the main dependent variables of interest. Results: The muscarinic receptor antagonist scopolamine improved decision-making overall, decreasing selection of one of the risky options while increasing choice of the more advantageous options. The muscarinic agonist oxotremorine increased choice latency but did not significantly affect option preference. Neither the nicotinic antagonist mecamylamine nor the agonist nicotine affected choice patterns, but mecamylamine decreased premature responding, an index of motor impulsivity. Conclusions: These results contrast sharply from those obtained previously using the uncued rGT, and suggest that the deleterious effects of win-paired cues on decision-making and impulse control may result from elevated cholinergic tone.Declaration of interest/funding. The authors confirm they have no conflicts of interest or financial disclosures to make.

Sex differences and reductions in open-field variable reliabilities and intercorrelations after immunogen exposure in adolescent rats

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Introduction. The open field (OF) is an apparatus commonly used in rodent behavioral studies. Here, we examined the sex differences in the reliabilities and intercorrelations of OF locomotor and anxiety-like behavioral measures and the effects of early adolescent immune stress using lipopolysaccharide [LPS] on OF variable reliabilities and cross-correlations in later adolescence. Predictions. (a) Aggregation increases reliabilities across test days, (b) exposure to LPS decreases reliabilities and intercorrelations (c) and effects would be more pronounced in females than males. Methods. Long Evans male (n=32) and female (n=32) rats were injected with LPS (200 µg/kg) or vehicle control (VEH) on postnatal day (PND) 30 and 32. Locomotor and anxiety-like behaviors were measured in an OF on PND 38-40. Locomotor variables included total horizontal distance moved (TD), number of horizontal movements (NHM), distance per horizontal movement (D/HM) and number of vertical movements (NVM). Anxiety-like variables included central duration (CD), central nosepokes (CNP), number of peripheral to central OF transitions (NT) and thigmotaxis ratio (THIG). Results. High positive correlations were found for locomotor and anxiety-like behavioral measures from PND 38-40 (range=.401-.842). Aggregation across PND 38-40 increased these scores substantially (range=.723-.945). When aggregated OF variable intercorrelations were examined, male rats had significantly reduced correlations for the LPS vs the VEH group for locomotor variables correlated with NHM (especially CNP and NT) and D/HM (with CD and NT). In females, significant differences were found for the LPS vs the VEH group in THIG with NVM and CNP, and TD and NVM. Discussion. In line with our predictions, aggregation increased the reliability of locomotor and anxiety-like measures. LPS exposure decreased intercorrelations for some locomotor measures. Contrary to our predictions, LPS-related changes were more pronounced in males than females. As such, the OF locomotor and anxiety-like behavioral measures are reliable. However, when rats are exposed to an immunogen, such as LPS, intercorrelations, especially of locomotor measures in male rats, are reduced. Funding. Natural Sciences and Engineering Research Council (NSERC) of Canada

Global and local strategy use by rats in the Traveling Salesman Problem.

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The Traveling Salesman Problem (TSP) is an optimization problem in which the goal is to find the shortest possible route that passes through each of a set of spatial targets. The TSP is of interest not only in the fields of mathematics, computer science and engineering, but also in cognitive and behavioral research to study problem solving and spatial navigation. Humans are able to complete even complex TSPs with a high degree of efficiency, and distance minimization in TSP analogues has been observed in a variety of non-human species as well. The TSP also has potential for translational research on cognitive and neurological disorders such as Alzheimer's Disease. For this reason, we are especially interested in the strategies used by rats in the TSP. This experiment was designed to further examine those strategies. After pre-training for the task, rats were tested once on each of several target configurations, and their travel routes recorded. We examined the routes for evidence of two local strategies (nearest neighbor and crossing avoidance), and one global strategy (tracing the convex hull). Our results indicated that fewer than 50% of transitions were between nearest-neighbor targets, indicating that this was not the dominant strategy, although use of this strategy increased in configurations with more targets. Subjects also exhibited clear avoidance of path-crossing; while crossing-avoidance is considered a local strategy, it also arises as a result of traveling along the convex hull, which is a global strategy. More than 50% of transitions, and significantly more transitions than predicted by chance, were along the hull of the configurations. Taken together, our results suggest that the performance of rats cannot be predicted by the nearest-neighbor strategy. Results are consistent with the use of either a crossing-avoidance or convex hull strategy with less complex configurations, with a slight increase in the use of nearest-neighbor as target complexity increases.

The role of mu-opioid receptors in the reinstatement of responding to an alcohol-predictive cue.

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We developed an animal model of relapse that examines how exposure to alcohol impacts the future reinstatement of responding to an extinguished alcohol-predictive cue. Here, we examined the role of mu-opioid receptors in this reinstatement effect. Rats (Experiment 1: males; Experiment 2: females and males; Long Evans, Envigo) received either 15 sessions of intermittent access to 15% alcohol (Experiment 1) or one session of habituation to 10% sucrose (Experiment 2) in the home-cage. Subsequently, rats underwent Pavlovian conditioning to associate a conditioned stimulus (CS; 20 s white-noise) with an alcohol (Experiment 1) or sucrose (Experiment 2) unconditioned stimulus (US) that was delivered into a fluid port for oral intake (0.3 mL/CS). Next, extinction was conducted, during which the CS was repeatedly presented without the US. Rats were then re-exposed to the alcohol- or sucrose-US as during Pavlovian conditioning, but without the CS. A reinstatement test was conducted 24 h later, during which the CS was presented in the absence of the US. Prior to the reinstatement test, rats received a systemic injection of the mu-opioid receptor antagonist, naltrexone hydrochloride (0, 0.3 or 1.0 mg/kg; 1.0 ml/kg). In Experiment 1, 1.0 mg/kg naltrexone reduced reinstatement of port entries to the alcohol-CS, but neither 0.3 mg/kg nor saline affected reinstatement. In Experiment 2, neither dose of naltrexone affected reinstatement of port entries to the sucrose-CS. Interestingly, females showed overall greater reinstatement compared to males. After additional Pavlovian conditioning, a higher dose of naltrexone (3.25 mg/kg) significantly reduced port entries to the sucrose-CS during an extinction session, suggesting that a higher dose of naltrexone may be necessary to reduce conditioned responding to a sucrose-CS. Our data show that the persistent effect of alcohol on future responding to an alcohol-cue is diminished by a mu-opioid receptor antagonist, and that this is an alcohol-specific effect. Our next experiments will investigate if mu-opioid receptors in the ventral hippocampus are required for reinstatement. Funded by the Canadian Institution of Health Research and the Fonds de Recherche du Quebec Nature et Technologies.

Analgesic doses of opioids induce gait alterations in mice.

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Mice are widely used to study the behavioral effects of opioids, including analgesia and dependence. Anti-nociceptive properties of opioids are often tested in uninjured mice using reflexive hot plate and tail flick assays. To study analgesia in injured mice, scientists evoke pain responses by directing noxious stimuli to the site affected by the injury, which is often the hind paw. However, applying stimuli to the hind paw in an opioid-treated mouse is challenging due to opioid-induced increases in locomotor activity. We aimed to overcome this challenge by using gait analysis to observe hind paw usage during walking in mice. We measured changes in paw print area following induction of post-surgical pain (using the paw incision model) and reversal with oxycodone. Paw incision surgery resulted in less weight bearing on the incised section of the paw and a reduction in the paw print area of the injured hind paw. Oxycodone caused a tiptoe-like gait in mice, resulting in a reduced paw print area in both hind paws. Further investigation of this opioid-induced phenotype revealed that analgesic doses of oxycodone or morphine dose-dependently reduced front and hind paw print area in uninjured mice. Interestingly, the gait changes caused by opioids were not caused by increases in locomotor activity; speed and paw print area had no correlation in opioid-treated mice, and other analgesic compounds that alter locomotor activity did not affect paw print area. Unfortunately, the opioid-induced â€œtiptoeâ€• gait phenotype prevented gait analysis from being a viable metric for demonstrating opioid reversal of pain sensitization in injured mice. These results suggest that scientists should use caution when using hind paw-directed nociceptive assays to test opioid analgesia in mice. Additionally, this study adds to the literature on opioid-induced muscle rigidity, providing evidence of how that stiffness affects function by impacting walking activity in mice. Therefore, our characterization of how opioids affect gait has important implications for the use of mice to study opioid pharmacology. National Institute of Neurological Disorders and Stroke (R01 NS042595), National Institute of General Medical Sciences (T32 GM108539)

The neurophysiological consequences of acute stress exposure on promoting a sedentary lifestyle.

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The World Health Organization lists physical inactivity as the 4th leading risk factor for global mortality. Therefore, identifying the factors that contribute to a reduced willingness to be active may be central to the development of new approaches that increase physical activity. While a growing body of evidence implicates psychological stress as a contributing factor to the sedentary lifestyle, little is known about how stress exposure may alter the brain in a manner that makes individuals more prone to physical inactivity, especially long after the stressor is no longer present. We have found that rats exposed to a single episode of uncontrollable tail shocks (acute stress), 36 hrs before being granted access to running wheels, display at least a two-month decrease in motivation to engage in physical activity, as revealed by a potently reduced daily wheel running distance. Moreover, running deficits cannot be explained by changes to eating or body weight following acute stress. Here, we test the hypothesis that running deficits are related to a maladaptive brain response to stress, that elicits early onset central fatigue and deficits in motivation. Therefore, young adult male Sprague Dawley rats were trained to run on treadmills at a mild speed. Rats were then exposed to acute stress or left undisturbed in home cages. Ten days after stress, rats were run until exhaustion on treadmills. Our results show that rats exposed to stress run approximately 10 min less than non-stressed rats, suggesting an earlier onset of fatigue. A separate cohort of stressed and non-stressed rats then ran until exhaustion on treadmills ten days after stress but were sampled at the average time point that stressed rats reached fatigue. Using uHPLC, ratios of 5HT:DA turnover were measured across the brain as a marker of central fatigue. Markers of muscle fatigue were evaluated in the gastrocnemius and soleus muscles using western blot. Data collection is underway, however, we hypothesize that markers of central fatigue will be elevated, in the absence of muscle fatigue, for stressed rats as compared to non-stressed rats, providing evidence that acute stress induces persistent early-onset fatigue that is related to running deficits.

Social dominance protects against the behavioral outcomes of inescapable stress

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An individual's social status has profound implications for health and well-being, with low status associated with greater prevalence of stress-linked disorders such as anxiety. However, there has been relatively few studies that have investigated the impact of dominance status on stressor outcome, and whether this process differs between the sexes. To investigate this relationship, triads of male and female Sprague-Dawley rats were exposed to five sessions of a novel social competition task (warm spot test). Stable winners and losers were revealed following the warm spot sessions and were subsequently exposed to stress treatment: inescapable stress (IS) or no stress (home cage, HC). Twenty-four hours later, social interaction with a juvenile conspecific was assessed. Male winners were protected against typical IS-induced social avoidance. This protection was absent in females. These results suggest that dominance status provides stress resilience in a sex-dependent manner. Funding acknowledgements: R01 MH050479 (MVB), R21 MH116353 (MVB), R01 MH108523 (MGF), University of Colorado Boulder Undergraduate Research Opportunities Program (PTC)

Multi-site electrophysiological recordings in the basal ganglia during hedonic feeding.

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Obesity is a growing public health concern due to its increasing prevalence and because it is associated with a variety of disabling and fatal health conditions. Though several physical, psychological and social factors account for the development of obesity, caloric intake above the physiological needs is one of its major causes. Hedonic motivation for highly palatable foods drives humans and animals to overeating, and it is mediated in part by the nucleus accumbens (NAc) and connected brain nuclei. The NAc receives multiple glutamatergic inputs, mainly from the prefrontal cortex (PFC), basolateral amygdala (BLA) and ventral hippocampus (vHip). Studies in humans and animal research have posed that the NAc is a central regulator of hedonic feeding and is also a major site of dysfunction in obesity. However, the relative importance of NAc inputs in the regulation of hedonic feeding, as well as its involvement in obesity, remains unclear. Here, we recorded local field potentials simultaneously in the NAc and its three major inputs â€“the PFC, the BLA and the vHip- while mice received intra-oral infusion of sucrose solutions. This analysis revealed a synchronous increase in the power of the low gamma band between the PFC, BLA and NAc, locked to the liquid infusion. However, the size of these responses did not change with the concentration of sucrose. Though this signaling does not reflect the hedonic value of the liquid infused, we evaluated if low gamma synchrony predicts variations in weight gain. Our data suggest that the level of synchronicity between the PFC and the NAc at the low gamma band correlates with the weigh gained after one week of high fat diet. Altogether, our preliminary results indicate that synchronous oscillatory activity at the low gamma band between the NAc and its inputs, and in particular with the PFC, reflex the occurrence of food delivery and may predict weight gain.Funding acknowledgements: Washington University in Saint Louis, School of Medicine

The medial pre-frontal cortex to lateral hypothalamic circuit is necessary for stress-induced inhibition of feeding behavior.

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The medial pre-frontal cortex (mPFC) is a key brain region implicated in food reward valuation. Human imaging studies demonstrate that both obese and anorexic patients have altered mPFC activation in response to food cues, suggesting a broad role for the mPFC in disordered feeding. Specific mPFC circuits to the limbic region and thalamus regulate different aspects of feeding behavior and reward seeking, however projections from the PFC to the hypothalamus have not been examined. Here, we describe a role for the mPFC to lateral hypothalamus (LH) circuit in the top-down control of feeding behavior. To determine the activity dynamics of the mPFC-LH circuit we used a dual-viral approach to express the fluorescent calcium indicator GCamp7s in the mPFC to LH projection neurons of C57BL/6 mice. Using fiber-photometry, we observed that exposure to acute stressors reliably increased the activity of the mPFC to LH projection neurons. We then targeted hM3Dq or hM4Di designer receptors exclusively activated by designer drugs (DREADDs) to the mPFC-LH projection neurons of C57BL/6 mice and acutely activated or inhibited this circuit using clozapine-N-oxide (CNO) prior to assessing feeding and reward-seeking behaviors. DREADDs-mediated inhibition of this circuit did not affect feeding or reward-seeking in a home cage environment. However, when feeding behavior was examined in a novel environment or in response to a novel food, inhibition of the mPFC-LH circuit significantly increased food intake, suggesting this circuit is required to inhibit feeding in response to stressful stimuli. Supporting this, we observed that DREADDs-mediated activation of this circuit is sufficient to suppress food intake and reduce motivation to obtain a sucrose reward. Taken together, our results suggest the mPFC-LH circuit senses stressful stimuli and is necessary for stress-induced suppression of feeding behaviors. These findings reveal a potential role for the mPFC-LH circuit in disorders related to stress and feeding behaviors.

Alzheimer’s disease-like network disruptions in hippocampus and anterior cingulate cortex in a model of diabetes mellitus.

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Network function in the hippocampus (HC) and anterior cingulate cortex (ACC) is critical for efficient working memory and network impairments play a significant role in the cognitive deficits seen in Alzheimer's disease (AD). Chronic hyperglycemia leads to neuroinflammation and tau hyperphosphorylation in the HC, similar to what occurs in AD, though with no amyloidosis. Interestingly, chronic hyperglycemia also leads to mild cognitive impairments, as observed in diabetes mellitus, a risk factor to the development of AD. We hypothesize that hyperglycemia is impairing HC network activity and cognitive performance, perhaps due to neuroinflammation and tau hyperphosphorylation. To test this hypothesis, we injected rats with a series of acute, low doses of streptozotocin (STZ) that damaged pancreatic beta cells and increased blood glucose levels. We then tested animals on a spatial delayed alternation task while simultaneously recording from the HC and ACC, two areas where network activity is essential for effective performance. We found that, as delays increased, hyperglycemic animals had a decrease in accuracy to chance levels. Spectral changes observed in STZ animals showed a decrease in HC theta power and an increase in delta power in both areas. This increase in delta power resulted in an overall change in the theta/delta ratio that was more noticeable during the end of the delay period, where theta power should be more pronounced. Interestingly, STZ animals showed strong hypersynchrony, with an increase in cross-frequency coupling, in several different frequency bands, and HC-ACC coherence. The elevated coherence significantly decreased during correct trials in STZ animals compared to the opposite effect seen in controls and previous research. Finally, we found a considerable amount of hyperphosphorylated tau within the HC and ACC of STZ animals, which may be related to the oscillatory and behavioral changes seen here. Collectively, these findings display striking changes in HC and ACC network activity during a working memory task in moderately hyperglycemic animals, suggesting an association with the physiological and cognitive pathologies of AD.

The effect of cannabigerol on the motivation and relapse to methamphetamine.

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The psychostimulant methamphetamine (METH) is a highly addictive illicit drug associated with physical and mental health problems. Pharmacological approaches for METH dependence suffer limitations due to poor efficacy. Cannabis constituents demonstrate therapeutic potential because of their anxiolytic and neuroprotective properties. We have previously shown that cannabidiol (CBD) reduces the motivation and relapse to METH seeking behaviour. Cannabigerol (CBG) displays emerging therapeutic properties. As such, we aimed to test the ability of CBG to reduce motivation and relapse for METH in rat models of chronic drug use. Thirty-six male Sprague Dawley rats with implanted jugular vein catheters were initially trained to self-administer METH via lever press during two-hour sessions on a fixed ratio 1 schedule of reinforcement. Of these, 16 rats advanced to a progressive ratio schedule to examine the effects of CBG (20, 40, and 80mg/kg) on reward motivation. We tested a combination of CBG+CBD (20+80mg/kg), to determine if it would inhibit reward motivation, using a known effective dose of CBD (80 mg/kg) as control. The remaining 20 rats advanced to extinction phase and subsequent methamphetamine-primed relapse tests with treatment injections of CBD 80mg/kg, CBG 20mg/kg, CBG+CBD (20+80mg/kg). None of the doses of CBG tested reduced the motivation to self-administer METH. As expected, CBD administration reduced the motivation to self-administer METH, however, the co-administration of CBG reversed this effect. A similar pattern of effect was shown with relapse to METH, where CBG and CBD+CBG had no effects on relapse, however treatment with CBD reduced METH-seeking behaviour. This is the first study to analyse the effect of CBG treatment on METH seeking behaviour and suggests that, at least at the doses tested, CBG has a greatly different pharmacology than CBD, and potentially could be antagonizing the effect of CBD to reduce the motivation for and relapse to METH taking. Funding acknowledgement Lambert Initiative for Cannabinoid Therapeutics and Macquarie University.

Graph-metrics-based brain connectivity distinguishes different modalities of semanticprocessing.

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The analysis of orthographic errors in transparent languages is crucial to understand the neural basis of reading processes. In this context, the study of the brain connectivity underlying semantic decision tasks involving spelling errors might help reveal recognizably distinctive functional processing patterns. With this central aim, thirty healthy young adult participants with more than 12 school years (15 male and 15 female) sequentially read 6-word sentences in which the last word determined the sentence to be: 1) congruent, 2) congruent with a pseudohomophone error, 3) congruent with a homophone error, 4) incongruent, or 5) incongruent with a pseudohomophone error. Twenty-seven sentences by condition were included, with simultaneous EEG recording. Twenty-five artifact-free EEG epochs -lasting 1300 ms each- were selected for analysis per condition, using Laplacian filter to cope with the volume conductor effects. The coherence matrix for each condition was obtained by averaging the different frequency bands, and they were all represented by a graph. Later, the metrics of degree, betweenness centrality, and closeness centrality were obtained for each of the bands of the average matrices of symmetric differences. The results show significantly different connectivity patterns depicting each analyzed condition. Moreover, a clear distinction between brain connectivity underlying the two closing types of orthographic errors (homophones and) was found. The present results seem to support the notion of a general neurophysiological arrangement devoted to error detection that goes far beyond the detection of semantic mismatches, involving the on-line pondering of the error's nature. FUNDING: Universidad de Guadalajara.

Discovery of the neural mechanisms of action of a novel treatment for opioid withdrawal.

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Opioid misuse is an escalating crisis accounting for 3 deaths per day in Australia and is now the #1 cause of preventable deaths in the USA. Repeated use of the prescription opioid oxycodone rapidly produces dependence, whereby when use is ceased, profound withdrawal symptoms are experienced. This withdrawal syndrome can be relieved by taking the opioid again, causing the individual to lose control over their opioid use, which may result in difficult to treat opioid use disorder. It is essential to treat withdrawal symptoms before this cycle begins. Unfortunately, there are few effective therapies for opioid withdrawal. We have developed a novel small molecule, KNX100, which potently reduces opioid withdrawal symptoms in mice exposed to naloxone-precipitated withdrawal after 8 days of oxycodone injections (twice-daily, increasing doses from 9 to 33 mg/kg). Our goal was to determine KNX100's mechanism of action in the brain. We first conducted whole-brain analysis of neuronal activity using cFos immunofluorescence, to identify regions where KNX100 reduced withdrawal-induced activity. This identified the nucleus accumbens shell (NAcSh), which is a key driver of opioid withdrawal. Next, we used fibre photometric measurement of jGCaMP7s-expressing cells in the NAcSh and discovered that withdrawal-induced jumping was temporally associated with increases in NAcSh activity, and that KNX100 reduced this jump-related activity, as well as reducing elevated baseline and maximal NAcSh activity during withdrawal, demonstrating a likely causal relationship between NAcSh activity and withdrawal-jumps. We then used chemogenetics to inhibit the NAcSh, which was sufficient to reduce withdrawal-induced jumping. Last, we injected KNX100 directly into the NAcSh, at a free drug concentration which is achieved in the NAcSh after systemic KNX100 dosing, which recapitulated the effects of chemogenetic NAcSh inhibition. Together, these data strongly indicate that KNX100 acts via the NAcSh to reduce opioid withdrawal symptoms. Future studies will characterise the cell types which KNX100 interacts with to reduce opioid withdrawal. This work was funded by a research contract with Kinoxis Therapeutics Pty Ltd, and by the National Institute On Drug Abuse of the National Institutes of Health.

Neural compensation in a novel, operant devluation task

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Deficits in goal-directed action are reported in multiple neuropsychiatric conditions. However, dysfunction is not always apparent , possibly due to neural compensation. We designed a novel devaluation task in which goal-directed action could be guided by stimulus-outcome (S-O) [presumably orbitofrontal cortex (OFC)-mediated] or response-outcome (R-O) associations [presumably prelimbic cortex (PL)-mediated]. In Exp 1, rats received bilateral OFC, PL, combined OFC+PL, or sham lesions and then completed our devaluation task. Sham, OFC, and PL lesioned rats showed intact devaluation, whereas the OFC+PL lesion group exhibited impaired devaluation. Next, we sought to determine whether the S-O strategy is used when the R-O strategy cannot be learned. In Experiment 2, rats received bilateral PL or sham lesions and then completed behavioral training. During testing, rats were divided into Cue Normal or Cue Switch test conditions. In Cue Switch test conditions, the cuelight and spatial lever location predicted conflicting outcomes so we can determine whether rats are devaluing using a S-O or R-O strategy. All groups exhibited normal devaluation except for PL Cue Switch condition rats that exhibited a reverse devaluation effect suggesting these rats were devaluing using an S-O strategy. Finally, we sought to determine whether the mediodorsal thalamus (MD) may be involved in this compensatory response. In Experiment 3, rats received unilateral infusions of cholera-toxin-b (CTb) into OFC and PL or sham lesions and then completed behavioral training. Rats were sacrificed on the last day of training to double-label for Arc and CTb in MD and Arc in the OFC. We found increased Arc+CTb in MD when PL is lesioned and increased Arc+ neurons in OFC when PL is lesioned. Our results suggest that our devaluation task can successfully model neural compensation between OFC and PL and this compensation may be regulated by MD. This research demonstrates a method to study how functional neural circuitry is subtly altered.This work was supported by the National Institutes of Health [grant number P20 GM113109-01A1 (C.L.P.)], Psi Chi (H.F.), the Histochemical Society (H.F.), Kansas State Graduate School (H.F.), and Sigma Xi (H.F.).

Automated and experimenter-free assessment of cognition and behaviour in rats: Introducing the PhenoSys sorting system.

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Animal models of psychiatric disease are essential for revealing causal relationships between specific biological mechanisms and human pathologies. It is now well-appreciated that repeated intervention and idiosyncratic handling techniques can have a dramatic influence on experimental outcomes in animal models. Therefore, the impact of the experimenters themselves can hamper reproducibility within and between laboratories. In order to effectively utilise animal models, it is important to reduce experimenter influence on behavioural outcomes. To this end, we exploited a fully-automated modular behavioural testing system from PhenoSys (Berlin) that includes home-cages and touchscreen operant testing chambers in order to assess pairwise discrimination and reversal learning. Female Sprague-Dawley rats (n=30) were implanted with subcutaneous RFID transponders so that an automated ID Sorter could allow selective passage between a group-living and testing chamber without experimenter intervention. We found that compared to conventional touchscreen testing, the rate at which rats acquired reversal tasks in the PhenoSys increased approximately 5-fold. Because animals can enter the touchscreen chamber at any time of the day or night when they are curious and motivated to perform, automation also unmasked variability in learning styles among animals. The continuous monitoring of animals using RFID technology also allows deeper consideration of behavioural complexity and to match home-cage behaviour with specific aspects of task performance. The broader adoption of automated behavioural testing systems that eliminate the need for experimenter intervention will likely improve both the reliability of behavioural assessment in rats and mice and reproducibility of findings within and between laboratories. We believe this represents a new frontier in behavioural neuroscience and a shift in the way the way these experiments are conducted and interpreted. We acknowledge the Rebecca L Cooper Medical Research Foundation for funding to CJF.

Determining the behavioral signatures of inflammatory pain at millisecond resolution.

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Chronic pain accounts for about 20% of physician visits and up to two thirds of patients are unsatisfied with current treatments. Much of our knowledge on pain and analgesia comes from behavioral studies performed on non-human primates and rodents. While humans can describe their pain levels both qualitatively and quantitatively, studies on rodents have for too long relied on assays providing incomplete behavioral information and often altering animals' original sensitivity via repetitive stimulation. Discovering novel analgesics therefore depends on our ability to provide more objective and comprehensive measurements of pain in laboratory animals. Using high-speed videography and automated paw tracking, we defined pain-related behaviors in mice. PAWS (Pain Assessment at Withdrawal Speeds) uses a univariate projection of hind paw position over time to automatically quantify 7 predefined behavioral features that we combined into a univariate pain score. While we previously described the behavioral signatures of acute evoked pain, the signatures of inflammatory pain - beyond paw withdrawal threshold & latency - remained elusive. Using PAWS in mice undergoing inflammatory pain, we separated pain-related and unrelated behaviors and successfully uncovered novel behaviors testifying for the conscious perception of carrageenan-induced pain in mice. Taken together, our methods provide a framework to rigorously assess pain-related behaviors in multitudes of pain paradigms.

Effect of growth mindset on cortisol and stress regulation in older adults.

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People with growth mindset of intelligence believe that their intelligence can be developed or cultivated while people with fixed mindset view their intelligence as something that cannot change. This is the essence of the implicit theories developed by Carol Dweck. Research has found a beneficial effect of growth mindset on academics. Furthermore, mindset has been shown to affect anxiety, attitudes under stressful situations, and behavior. However, to our knowledge, there has been no research on the effect of mindset on stress regulation in older adults. The goal of our study is to investigate if growth mindset predicts better psychological and physiological stress regulation in older adults. We tested male and female subjects 65 years of age and older living in independent residential and assisted living communities. Subjects went through a modified version of the Trier Social Stress Test requiring them to formulate and to give a speech followed by a relatively difficult arithmetic test. Saliva cortisol samples combined with a mood test were collected before the social stressor and following the procedure at various intervals. Subjects also took tests measuring implicit theories of intelligence and implicit theories of thought, emotion and behavior. Finally, subjects took the Depression, Anxiety and Stress Survey (DASS), the Self-Administered Gerocognitive Examination (SAGE), and an aging perceptions questionnaire. Our process of data collection was interrupted by the pandemic. We have so far collected data from 14 subjects and we hope to resume data collection once the institutions give us back the access to their residents. Based on results from the same study we carried out with college students, our hypothesis is that having more of growth mindset of intelligence and/or growth mindset of behavior will yield relatively better stress regulation. We ran regression analysis with the data we have so far and already found that implicit theories of intelligence significantly predicted a change in the subjects' mood following social stress. We also found that implicit theories of thoughts significantly predicted change in subjects' level of cortisol also following social stress.

Effect of working memory load on functional connectivity in type 1 diabetes.

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Cognitive deficits have been demonstrated to occur during Type-1-Diabetes (T1D) development. Working memory has been one of the most affected domains. Previous studies have mainly reported differences on task-related brain activation patterns in T1D patients compared to healthy controls on visuospatial working memory (VSWM) tasks. However, VSWM load on functional brain connectivity during task performance has not been studied in this population. The present study aimed to assess eighteen well-controlled T1D young patients and healthy matched controls performing a VSWM task with different memory load levels during an electroencephalographic recording. Functional connectivity index was estimated during two main VSWM processing phases: encoding and maintenance. The results showed that T1D patients had lower behavioral performance and longer reaction times compared to the control group. However, higher and progressively increasing functional connectivity indices were found in T1D patients since the beginning of the VSWM encoding phase, but not affecting the maintenance phase. In contrast, healthy controls managed to solve the task, showing lower functional brain connectivity during the initial VSWM processing steps with more gradual task-related adjustments. Moreover, functional connectivity was significantly different between groups in all of the load levels, particularly during the encoding phase. These results suggest that T1D patients anticipate higher task load demands by early recruiting excessive cognitive resources via increasing brain connectivity. Funding: Universidad de Guadalajara

The Cocaine and Oxycodone Biobanks: Two repositories of biological samples from genetically characterized outbred rats that exhibit compulsive-like escalation of cocaine or oxycodone self-administration.

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Identification of the mechanisms that underlie compulsive cocaine or oxycodone use in animal models is a major goal for understanding the genetic risk factors for addiction and facilitating the identification of novel druggable targets. A key issue for the field is the lack of a repository that contains biological samples from behaviorally and genetically characterized rats. We introduce the Cocaine Biobank (www.cocainebiobank.org) and the Oxycodone Biobank (www.oxycodonebiobank.org), two repositories of biological samples from a unique, genetically diverse strain of outbred heterogeneous stock (HS) rats that have been behaviorally and genetically characterized using next-generation sequencing, state-of-the-art behavioral screening, and a variety of preservation techniques. Male and female rats are trained to self-administer cocaine (0.5 mg/kg/inf) in daily 6 h sessions or oxycodone (0.15 mg/kg/inf) in daily 12 h sessions and tested using progressive-ratio responding, responding despite adverse consequences (contingent footshocks), and measures of analgesia, hyperalgesia and irritability-like behaviors. Results show high individual variability with vulnerable and resistant rats that is likely to facilitate the detection of gene variants and the molecular and cellular mechanisms of addiction. Preservation techniques include perfusion, snap-freezing, and cryopreservation to maximize the compatibility of these tissue banks with cellular, molecular, and anatomical methods. The Biobanks provides free access to over 20 organs. The Biobanks have the potential to facilitate the identification of novel druggable targets and provide a unique data/tissue repository that will facilitate follow-up and replication studies. Funding: This work was supported by National Institutes of Health grants DA044451 and DA043799 from the National Institute on Drug Abuse. The authors declare no competing financial interests.

The superiority of semantic over orthographic processing.

ERPs study. Gomez-Velazquez, Fabiola R. 1, Gonzalez-Garrido, Andres A. 1, QuiÃ±onez-Beltran, Juan F. 1, Ruiz-Stovel, Vanessa D. 1, Martinez-Ramos, Alicia 2, Gallardo-Moreno, Geisa B. 1. 1. Instituto de Neurociencias, Universidad de Guadalajara. 2. Departamento de Neurociencias, Universidad de Guadalajara.

Reading comprehension depends on the automatic recognition of written words through phonological and orthographic representation stored in long-term memory. Event-related potentials (ERP) studies have provided valuable information about semantic processing, showing that N400 is a component sensitive to expectancy violations. However, it is not clear how different types of word-properties are integrated during language comprehension. We used ERPs to study the interplay of phonological and orthographic information over semantic processing during reading in a group of 36 right-handed young adults (16 male). A semantic decision task of 170 six words sentences with high cloze probability were visually presented; participants were asked to determine if the 6th word of the sentence was semantically congruent or incongruent regardless of the possible presence of spelling errors. The closing word of each sentence was a) a congruent word, b) a congruent word with a pseudohomophone error, c) a congruent word with a typo-like error, d) an incongruent word or e) an incongruent word with a pseudohomophone error. Our results showed that N400 seems to be insensitive to orthographic information and is fully determined by semantic incongruency. Orthographic expectancy violations are reflected by a late positivity rather than a larger N400 amplitude. The P600 amplitude was modulated for pseudohomophones (sentence completions that are acceptable phonologically but are misspelled) and typo-like errors, but only in semantically congruent endings. It seems that orthographic information is not fully processed when there is deeper linguistic processing in progress, such as semantic incongruence. Furthermore, the participants' orthographic knowledge positively correlated with the amplitude of N400 and P600.

Fearful processing is disturbed by an early onset of type-1 Diabetes.

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Type-1-Diabetes (T1D) development has been related to cognitive dysfunction, especially when it has an earlier onset. Therefore, cognitive processes maturing later in neurodevelopment -as it occurs with the emotional processing of fearful facial expressions- tend to be more exposed to the illness's deleterious effects. The present work aimed to evaluate the pattern of neural activations (BOLD signal) of young T1D patients and paired healthy controls using functional magnetic resonance (fMRI) methods while performing an emotional recognition block-design task involving fearful and neutral facial expressions. Even though no significant differences were found between the groups in the number of correct responses and reaction times, they showed different brain activation patterns when processing fearful faces. The control group showed more significant activations in the angular and supramarginal gyri. In contrast, T1DM patients showed greater activations in the orbitofrontal cortex and substantial activations in brain structures related to neutral and fearful face processing that were not present in the control group. The current results confirmed that T1D affects the brain neurofunctional activation pattern associated with fearful facial processing. It suggests a lower level of automatization in fear perception processing.

GPR52 agonism reverses schizophrenia-relevant spatial working memory deficits in mice.

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Deficits in working memory (WM) constitute one of the most common and severe of cognitive impairments associated with schizophrenia (CIAS). While the severity of CIAS is the most accurate predictor of patient outcomes, there remains no antipsychotic able to improve cognition in patients. The orphan receptor, GPR52 is a prospective therapeutic target for the treatment of WM deficits in CIAS due to enrichment in brain regions critical for WM function. The pro-cognitive capacity of GPR52 agonists, however, has not been assessed using translationally-relevant behavioural tests. Herein, WM performance was assessed using the mouse touchscreen-based spatial WM task, TUNL (trial-unique, delayed nonmatching-to-location). To determine if the acute MK-801 NMDA receptor hypofunction model would produce deficits in TUNL, a dose-response relationship was established for task performance. We then used this MK-801 model to assess the pro-cognitive capacity of the GPR52 agonist, 3-BTBZ. Neuronal activation in key brain regions following treatment with MK-801 and 3-BTBZ was measured by c-fos induction. We found that 10 mg/kg 3-BTBZ reversed MK-801 (0.15 mg/kg)-induced WM deficits in TUNL and, in the absence of MK-801, improved performance over vehicle at high WM loads. 3-BTBZ reversed MK-801-induced c-fos induction in regions of the prefrontal cortex, nucleus reuniens, and entorhinal cortex although not in other brain regions such as the amygdala and hippocampus. These data suggest not only that the GPR52 agonist, 3-BTBZ has schizophrenia-relevant pro-cognitive efficacy, but can also improve cognitive performance in its own right in the absence of a disease model. This study further implicates activity in key nodes specifically involved in WM function and known to be dysfunctional in schizophrenia patients; the medial prefrontal cortex and nucleus reuniens, as a mechanism for GPR52-mediated pro-cognitive effects. Ultimately this study provides for the first time, preclinical validation for the use of GPR52 agonists in the treatment of WM deficits in schizophrenia using translationally-relevant measures.

Sex differences in the relationship between negative cognitive bias and neuroinflammation after chronic unpredictable stress in rats

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Major depressive disorder (MDD) is more common in women than in men, and women have more severe symptoms of MDD. Cognitive symptoms of MDD include negative cognitive bias. Negative cognitive bias is an increased perception of neutral situations or objects as negative. Elevated inflammation has been linked to MDD and cognitive impairment. MDD can be modelled in rodents using a chronic unpredictable stress (CUS) paradigm. We examined whether there were sex differences in cognitive bias after CUS and in neuroinflammation by examining nine cytokine concentrations in the ventral hippocampus and basolateral amygdala. Adult male and female Sprague-Dawley rats underwent either 2 weeks of CUS or no stress followed by an 18-day fear-based cognitive bias task. On day 1 of cognitive bias training, male and female rats that underwent CUS displayed a more freezing (greater fearful response) than controls. By the end of cognitive bias training (day 16), rats exposed to CUS displayed a potentiated fear response in all contexts compared to non-stressed rats, and males displayed more freezing than did females. CUS increased freezing in the ambiguous context. In addition, CUS increased positive relationships between ambiguous freezing and inflammation in the basolateral amygdala in females, and CUS increased negative relationships between ambiguous freezing and inflammation in the ventral hippocampus of males. These findings indicate that CUS potentiates the freezing response to a neutral context, increasing negative cognitive bias in both sexes and suggests a sex-specific role of neuroinflammation in negative cognitive bias. Funding Acknowledgement: CIHR MOP 142328 held by LAMG, WHRI Catalyst held by THE

Norepinephrine augments attention through phosphorylation of the L-type Ca channel Cav1.2.

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Norepinephrine (NE) and dopamine (DA) are critical regulators of attention. Drugs acting on NE and DA transporters in the brain are used to treat clinical inattention. However, the importance of specific signaling pathways and relative contributions of NE versus DA remain unknown. Previous reports suggested an association of NE signaling via beta-adrenergic receptors (beta1/2-AR) with the regulation of sustained attention measured by reaction time variability (RTV). In clinical settings and animal models, increased RTV has been interpreted to reflect distractibility or lapses in attention and is a key endophenotype of neuropsychiatric disorders that affect attention like ADHD, ASD, BD, MDD, and SCZ. Common risk factors for these disorders identified by GWAS are mutations in the genes that encode the most prevalent L-type Ca2+ channel, Cav1.2. We found previously that the beta2-AR forms a unique signaling complex with Cav1.2 that contains the full set of proteins required for cAMP signaling, including Gs, adenylyl cyclase and PKA (Science 293, 98-101). Because this is a unique signaling complex, we hypothesized that it constitutes a crucial target of NE signaling. The beta2-AR augments channel activity of Cav1.2 via its phosphorylation at residue Ser1928. Here, we report the new finding that mice that carry a serine-to-alanine knock-in mutation to specifically block NE signaling through Cav1.2 (S1928A KI mice) exhibit increased RTV relative to WT mice in the 5-Choice Serial Reaction Time Task when attention is challenged. Inhibition of NE reuptake by Atomoxetine and stimulation of the beta2-AR by Clenbuterol decreased RTV in WT, but not S1928A KI mice. Stimulation of cAMP-linked dopaminergic D1/5R with SKF38393 did not affect RTV in either genotype. These findings suggest that stimulation of Cav1.2 activity by NE via beta2-AR and Ser1928 phosphorylation plays a privileged role in the enhancement of sustained attention. These findings may help to define signaling dysfunction in mental disorders where elevated RTV is an important endophenotype. Our results indicate S1928A KI mice as a new animal model for attention disorders and point to regulation of Cav1.2 as a potential target for novel drug development.

Estradiol infused into the medial prefrontal cortex of female mice rapidly facilitates social recognition.

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Social recognition (SR) is integral for creating social structures that are essential in many social species, including mice. SR is influenced by various hormones including estrogens such as estradiol (E2). Within minutes of systemic E2 administration, SR is rapidly facilitated in female mice. Similar facilitating effects on SR are seen when E2 is infused into specific brain regions that comprise the social brain, including the hippocampus and medial amygdala. Another brain region with a significant role in social cognition is the medial prefrontal cortex (mPFC) which also expresses all three estrogen receptors. Therefore, we hypothesize that E2 in the mPFC mediates SR. In this project, sexually mature female mice were ovariectomized (to control for circulating estrogens) and had a cannula placed above their mPFC, to allow for direct drug infusions. These mice received an mPFC infusion of either E2, at 25nM, 50nM or 100nM, or a control of 0.02% ethanol in artificial cerebrospinal fluid, 15 minutes before mice take part in a social recognition paradigm that was designed to be too difficult for ovariectomized control mice to demonstrate SR. This paradigm consists of the experimental mouse being habituated to a pair of mice before taking part in a test phase where they are exposed to a familiar mouse (from the previous pair) and a novel mouse. The entire paradigm was completed within 40 minutes, to observe the rapid effects of E2 on SR. Since mice prefer novelty, if SR occurs, the experimental mouse should show a preference for the novel mouse during the test phase. Preliminary results suggest that E2 infused into the mPFC does rapidly facilitate SR. Mice that received 50nM of E2 spent significantly more time investigating the novel mouse during the test phase compared to the sample phase and compared to the control group. Overall, this research will provide us with novel information on the effects of estrogens within the social brain. Funding: This study was funded by the Natural Science and Engineering Research Council of Canada (NSERC)

Neuromodulatory effect of exogenous melatonin on Central Post Stroke Pain in rodents.

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Around 12% of stroke survivors suffer from a devastating chronic pain condition called central post stroke pain (CPSP). These patients may accompany cognitive deficit, depression and disturbed sleep pattern that make them vulnerable to be wrongly diagnosed and treated. Current treatments often provide little or no relief to CPSP patients. In the present study, we established a CPSP animal model by intra thalamic lesion by collagenase microinjection, which was assessed by mechanical and thermal planters and cold allodynia. After the rehabilitation period of three weeks, melatonin was administered for the next three weeks. A significant decrease in mitochondrial chain complexes and enzymes were observed after thalamic lesion. Proinflammatory infiltration was noticed after thalamic lesion. Administration of melatonin has been shown to reverse the injury effects dose dependently, while naloxone treated rats has been shown to block the antinociceptive effect of melatonin, suggesting involvement of opioid receptors too. A significant increase in mitochondrial complexes and enzymes in thalamic lesion rat brain was found. Further the proinflammatory cytokines were significantly reduced by melatonin treatment. Moreover, the agonizing pain due to CPSP comes with disturbed circadian activity as comorbidity. We have seen differences in light and dark activity behavior in rats due to CPSP. For 3weeks 30mg/kg chronic melatonin administration, helped in restoring all the hampered activity behaviors in rats due to CPSP during both light and dark periods and the persisted effect was evident after discontinuing melatonin administration.Melatonin seems to mediate its actions through MT1/MT2 as well as mu opioid receptors. We speculate that involving these receptors, melatonin inhibits Adenyl cyclase and reducing cAMP, and thence reducing pain perception. Hence according to our study, exogenous melatonin represents a promising new neuromodulatory drug for pain alleviation in CPSP condition. Thereby, we further prospect the clinical utilization of melatonin in CPSP patients suffering from pain and sleep disturbance.Acknowledgment: Ministry of Science and Technology, Taiwan

The integration site of two olfactory systems upon the detection of the alarm pheromone in rats.

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Many animals are known to emit an olfactory signal called the alarm pheromone that warns other conspecifics nearby of danger. We have previously shown that the alarm pheromone in rats is composed of hexanal and 4-methylpentanal. When hexanal and 4-methylpentanal are detected simultaneously through the main olfactory system and the vomeronasal system, respectively, these substances induce the activation of the anxiety circuit, including the bed nucleus of the stria terminalis (BNST). However, little is known in regards to the integration of the information perceived through the two different olfactory systems. Here, we examined the Fos expression in 17 brain sites to determine the integration site of these separate neural pathways upon the detection of the alarm pheromone. Rats were exposed to either water (control), hexanal, 4-methylpentanal, or the mixture of the two substances in their home cage. Then, we observed Fos expression in sites that are involved in the two olfactory systems or the anxiety circuit. We found that Fos expression was increased in the posteroventral medial amygdala (MePV) in the hexanal, 4-methylpentanal, and mixture groups compared to the control group. In addition, the MePV showed higher Fos expression in the mixture group compared to those in the 4-methylpentanal group. We also found that only the mixture showed increased Fos expression in the anterior bed nucleus of the stria terminalis (BNSTa), anteroventral medial amygdala, and the paraventricular nucleus of the hypothalamus. Furthermore, rats presented with the mixture showed increased Fos expression in the bed nucleus of the accessory olfactory tract and the posterior bed nucleus of the stria terminalis compared to rats presented with water and hexanal. Based on these findings, we suggest that the MePV is the integration site of information from the two separate olfactory systems after the detection of the alarm pheromone. Then, the information may be transmitted to the BNSTa and activate the anxiety circuit. This study was supported by JSPS KAKENHI (Grant Numbers 20H03160 and 20H04766).

Time Following Initial Acquisition is Sufficient to Make Signaled Active Avoidance Dependent on the Retrosplenial Cortex

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Signaled active avoidance (SAA) involves a multistage learning process supported by a shifting neural substrate. Although a good deal of work has explored the circuitry by which SAA is acquired and expressed, relatively little is known about the mechanisms underlying the long-term maintenance of the avoidance response, and even less is understood about the process by which these mechanisms are recruited. Because of its established role in long-lasting forms of aversive memory, we hypothesized that the retrosplenial cortex (RSC) is necessary for continued maintenance of SAA after substantial training. In an initial experiment, rats received intra-retrosplenial infusions of an AAV containing the gene construct for either the inhibitory hM4Di DREADD or GFP on a CamKII promoter. Following recovery, subjects were trained in an SAA paradigm in which they learned to shuttle across a divided chamber during a tone in order to avoid a foot shock. To compare the effects of RSC inactivation across different phases of SAA maintenance, subjects underwent four days of initial acquisition before receiving two test sessions preceded by CNO or vehicle in a counterbalanced order. Subjects then underwent two additional days of SAA training without drug treatment prior to two final test sessions that were again preceded by counterbalanced administration of CNO or vehicle. While CNO inactivation of RSC had no effect on the avoidance response following the initial four days of training, a robust decrement was observed at the latter time point, suggesting that RSC is necessary for avoidance after eight daily sessions of SAA training. Our subsequent experiment explored whether RSC is recruited to SAA by continued training following initial acquisition or by the passage of time, suggesting a systems consolidation-like process. Rats expressing hM4Di in RSC pyramidal neurons received either four days of SAA training before two test sessions (identical to those described above) or four days of SAA followed by four days of time off in the homecage prior to testing. CNO inactivation of RSC following time off caused a significant decrement in the avoidance response, yet inactivation immediately following the initial four days of training had no effect. Thus, our data demonstrate that RSC plays a role in the long-term maintenance of the avoidance response, and suggest the intriguing possibility that RSC is recruited to SAA by a systems consolidation-like process.

Engram size varies with learning and reflects memory content and precision.

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Memories are rarely acquired under ideal conditions, rendering them vulnerable to profound omissions, errors and ambiguities. Consistent with this, recent work using context fear conditioning has shown that memories formed after inadequate learning time display a variety of maladaptive properties, including overgeneralization to similar contexts. However, the neuronal basis of such poor learning and memory imprecision remains unknown. Using c-fos to track neuronal activity in male mice, we examined how these learning-dependent changes in context fear memory precision are encoded in hippocampal ensembles. We found that the total number of c-fos encoding cells did not correspond with learning history but instead more closely reflected the length of the session immediately preceding c-fos measurement. However, using a c-fos driven tagging method (TRAP2 mouse line), we found that the degree of learning and memory specificity corresponded with neuronal activity in a subset of dentate gyrus cells that were active during both learning and recall. Our work provides two surprising conclusions. First, engram size varies with learning. Second, larger engrams support better neuronal and behavioural discrimination. This work was supported by Boyarsky Family Trust, the Howland-Rose Foundation, Doug Battersby and family, David King and family, John Schaffer, Lady Fairfax Charitable Trust, Stanley and Charmaine Roth and we recognise Iain Gray in honour of Kylie. Funding was also provided by the NHMRC (grant 1083569) and the ARC (DP200102445) and NIMH RO1-62122 (MSF).

Intensity of cortical perineuronal nets after binge alcohol and/or physical exercise.

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Perineuronal nets (PNNs) are extracellular matrix structures that encapsulate the soma and proximal dendrites of neurons and are critical regulators of inhibitory signaling. Prior research indicates that binge alcohol increases PNN expression in the insular cortex whereas physical exercise decreases expression in several cortical regions. Here, we examined the effects of binge alcohol and/or exercise on PNNs in frontal cortical regions. We hypothesized that binge alcohol would increase, but exercise would decrease, cortical PNNs. We have previously shown that exercise normalizes binge alcohol-induced cell loss and neuroimmune activation in the hippocampus and cortex, suggesting that exercise counteracts binge alcohol effects. Therefore, we further hypothesized that PNN intensity in exercised binged animals would not differ from controls. In this study, we used fluorescein-conjugated Wisteria floribunda (WFA) to label PNNs in brain sections from adult female rats that were gavaged once-weekly with binge alcohol (5 g/kg) or isocaloric control diet, for 11 consecutive weeks. Half of the animals in each group exercised 3 days/wk. In 6 sections per animal, we traced cortical ROI at 2X, then acquired non-overlapping z-stacks at 20x using a confocal microscope and StereoInvestigator software. Fluorescent intensity of PNNs was quantified using ImageJ, combined with the PIPSQUEAK AI plug-in to standardize background subtraction. In partial support of our hypotheses, our data show that in the medial prefrontal, primary motor, cingulate and insular cortices there are interactive effects of binge alcohol and exercise. Consistent with other reports, we found that PNN intensity is region-specific, being highest in orbital frontal regions and lowest in medial prefrontal regions. To conclude, alcohol and exercise are common aspects of lifestyle and prior research shows that they exert competing influences on the brain. Our preliminary results build upon this body of research by showing that they also exert interactive effects on intensity of cortical PNNs, in a region-specific manner. Supported by NIAAA R01AA025380

Analyses of the Posterior complex of the Anterior Olfactory Nucleus Projections Associated with Rat Social Buffering.

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Social buffering is the phenomenon where stress responses are ameliorated by the accompanying conspecifics. We previously found that an accompanying rat activated the posterior complex of the anterior olfactory nucleus (AOP) of the subject rat, which in turn suppressed the basolateral complex of the amygdala (BLA) and induced social buffering. The preliminary study by c-Fos mapping suggested that the posteroventral medial amygdala (MePV) also participated in social buffering. However, the anatomic properties between these three areas remain unclear. Here, we analyzed efferent projections of the AOP toward the MePV and BLA. A retrograde tracer (Retrobeads) was infused either into the MePV or the BLA (n=5 each). Then, randomly selected five 40μm-thick coronal sections from Bregma 3.00 mm to 3.84 mm were observed in each subject. In the AOP, we found that the number of MePV-projecting cells (405.8 ± 23.5) was larger than that of BLA-projecting cells (243.6 ± 25.6). Among the MePV-projecting cells, 46.1 ± 6.9% and 21.7 ± 3.8% of cells expressed vGLUT2 and GABA mRNA, respectively. Similarly, 46.0 ± 10.3% and 18.6 ± 1.8% of BLA-projecting cells expressed vGLUT2 and GABA mRNA, respectively. In order to assess the existence of collateral projections to both the MePV and the BLA , another group of rats (n = 3) received two injections of retrograde tracers, one into the MePV and another into the ipsilateral BLA. Then, one of every three sections were observed. When we calculated the percentage of cell labeled by each or both tracers with respect to all the labeled cells, the percentage of collaterally projecting cells was small in the AOP (2.1 ± 2.1%). The percentage of MePV-projecting cells (74.4 ± 7.6%) was found to be larger than that of BLA-projecting cells (23.5 ± 5.5%). In addition, we confirmed that both types of projecting cells were evenly distributed from the rostral to caudal AOP. These results suggested that the AOP sends anatomically stronger projections to the MePV than to the BLA. Therefore, it is possible that the MePV receives signals responsible for social buffering from the AOP. This study was supported by JSPS KAKENHI (Grant Numbers 20H03160 and 20H04766).

The role of extended amygdala cell-types in a foraging task and threat response in mice

Ly, Annie; Root, David.

All organisms must find food for sustenance. When faced with a predatory threat while foraging for food, appropriate threat evaluation and response are paramount to survival. In these instances, organisms enact a foraging strategy and produce defensive behaviors appropriate to the threat, but maladaptive responses to a threat that is no longer present can be classified as anxiety. The activity of the extended amygdala, specifically the bed nucleus of the stria terminalis (BNST), is implicated in anticipatory threat response. However, the underlying relationship between extended amygdala activity and changes in foraging behavior in response to a threat experience is unclear. Using a combination of behavioral testing, fiber photometry, and in situ hybridization, the present work aimed to investigate the role of extended amygdala cell-types involved in foraging and threat assessment. We developed a semi-naturalistic foraging task with a simulated robotic land predator. Mice were trained to search for food at discrete and incrementally greater distances in a brightly lit arena. After obtaining food, mice learned to retreat into a dark, enclosed nesting zone. After observing stable response in foraging behavior, the mice were exposed to a robotic land predator positioned in front of a food source at the farthest distance. The robot was programmed to use infrared sensor detection to charge at a rodent and snap its mechanical jaws. When the food was positioned at the farthest distance from the nest, mice showed reductions in several foraging behavior parameters and adapt a path-efficient strategy after experiencing the robotic predator. Preliminary calcium imaging data suggest that specific cell-types within the BNST are sensitive to both feeding behavior and the motivation to forage. Additional tests of the causal influence these cell-types have on foraging are ongoing. Based on the results, we conclude that mice change foraging strategy after experiencing a semi-realistic predatory threat and that cell-types within the BNST may have a role in foraging behavior.

Interaction between gestational immune activation and environmental enrichment on ER-alpha and Iba-1 counts in the postpartum maternal rat hippocampus.

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Pregnancy exposes the maternal brain to a period of plasticity which is understood to be largely mediated by enrichment from exposure to new stimuli and changes to the maternal immune, endocrine and nervous systems to prepare for offspring care. Infections are a prevalent risk factor during pregnancy; however, the effects of gestational immune activation (GIA) on these systems and the maternal brain have not been fully characterized. In this work, GIA is used with respect to immune activation in the mothers, instead of the maternal immune activation (MIA) terminology that is typically used for offspring exposure. In addition to this, the protective effects of environmental enrichment against biological stressors such as an infection have mostly been investigated within the context of MIA offspring. This study examines the effects of lipopolysaccharide (LPS) induced GIA and housing conditions on the postpartum maternal hippocampus. Sprague-Dawley rat dams (NERalpha=32; NIba-1=19) were randomly housed in one of three conditions: standard housing (ACC), social enrichment (SE), or environmental enrichment (EE). On gestational day 11 they were treated with either LPS or saline vehicle (VEH). Treatment was found to have an effect on ERalpha counts within the CA3 region of the hippocampus ($F(1,26)= 6.94$; $p=0.014013$) with LPS treated dams having lower mean ERalpha counts than VEH treated dams; 9.99 ± 0.90 and 13.65 ± 0.94, respectively. No effects of housing and treatment or interaction between the two were observed for Iba-1 counts in the CA3 region of the hippocampus. These results demonstrate the potential for inflammatory processes during pregnancy to impact maternal brain plasticity, supporting the need for further research into the effects of GIA, especially in the era of SARS-CoV-2 outbreak.

Social motivation in the presence of genetic liabilities for autism spectrum disorder.

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Facets of social motivation are critical to the development and maintenance of healthy social functioning. Autism spectrum disorder (ASD) is defined in part by deficits in social communication and social interaction, yet a long standing hypothesis is that the root deficit may actually be in social motivation. Deficits in social motivation could be due to either a deficit in social reward circuits or social orienting circuits (i.e., failing to attend to a social stimulus when presented). We seek to understand if abnormalities in social motivation underlie the core social phenotypes in ASD. Thus we adapted standard operant conditioning to assess social motivation by rewarding nosepokes with an opportunity for transient social interaction. Social reward seeking is quantified by increasing the number of nosepokes required (work) to elicit each reward, and in parallel the animal's social orienting can be assessed by tracking its behavior. We tested social motivation function in two mouse models of ASD liability: Shank3b and Myt1l mutants. Shank3b mutants failed to work for access to a social partner by failing to exhibit an increase in nosepokes to elicit a social interaction. Whereas Myt1l mutants appeared work for access to a social partner by achieving a similar number of correct nosepokes to elicit a social interaction compared to controls, yet male mutants achieved fewer social rewards and spent less time in the social interaction zone. Together, these data suggest different aspects of social motivation are disrupted in these models. Shank3b mutants fail respond to social reward, while Myt1l mutant males failed to cease holepoking and attend to a social stimulus at the WT rate. This suggests SHANK3B loss may lead to ASD phenotypes by disrupting social reward circuits, while MYT1L loss might lead to ASD phenotypes by disrupting social orienting, possibly linked to cognitive inflexibility and inappropriate perseveration on non-social stimuli. Funding Support: The Jakob Gene Fund (JDD,SEM), NIMH (1R01MH107515-01A1, JDD; R01MH124808, JDD,SEM), and NICHD (P50HD103525, IDDRC@WUSTL).

Relationship between neighborhood poverty and children's externalizing behaviors as mediated/moderated by environmental and neurological factors.

Maxwell, Megan; Taylor, Rita; Barch, Deanna

Increased rates of internalizing and externalizing behaviors have been observed in children from more impoverished neighborhoods, and correlates of neighborhood poverty such as exposure to crime, toxin levels, or altered brain structure may contribute to this relationship. Additionally, receipt of socioemotional support as a protective factor can mitigate the impact of adversity on children’s developmental outcomes. The goal of this project is to examine the extent to which neighborhood poverty relates to children’s mental health outcomes independent of household socioeconomic status, and to determine whether toxin levels and brain volume serially mediate this relationship and whether socioemotional support moderates it. Data from the Adolescent Brain Cognitive Development study were obtained for 8,623 9-10 year old children; neighborhood poverty was measured using nine census tract variables from the Area Deprivation Index and mental health symptoms were assessed by the Child Behavior Checklist. Toxin levels included particulate matter, nitrogen dioxide, and lead risk measured by the NASA SEDAC while amygdala, dlPFC, and intracranial volumes were obtained by MRI. Socioemotional support consisted of parent, peer, and school sources assessed by various questionnaires. Generalized linear models and structural equation modeling tested for evidence of a mediation and moderation. Increased neighborhood poverty was significantly associated with increased externalizing symptoms, and reductions in intracranial volume mediated this relationship. Increased parental support as assessed by the youth-reported Family Environment Scale and Parental Monitoring Survey was also a significant moderator such that children lower in poverty exhibited fewer externalizing behaviors. These results highlight the importance of identifying environmental and neurological markers that may increase risk for later psychopathology in order to better inform holistic interventions designed to reduce the burden of mental illness.

Participation of autism associated MYT1L in motor function development.

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Mutations in the Myelin transcription factor 1-like (MYT1L) gene result in a syndrome associated with intellectual disability (ID), autism spectrum disorder (ASD), and attention-deficit hyperactivity disorder (ADHD), and characterized by universal speech and motor delay. To understand the role of MYT1L on normal motor development and function, we leveraged our novel loss-of-function mouse model that harbors a patient-specific MYT1L amino acid change to examine different aspects of motor function across the lifespan. During the first two weeks postnatal, we conducted a series of assessments in our model to examine possible motor delay as well as general development. In an independent cohort, we examined the development of gait in the absence of MYT1L during the juvenile stage and then assessed the persistence of gait features in mature animals. Finally, in a third cohort we examined strength, coordination, and balance abilities during adulthood. We found no signs of gross developmental delay in the Myt1l het mice; they achieved physical milestones and reflexes similarly to their WT littermates. However, the Het mice did show reduced latencies in tasks of muscle strength and endurance, suggesting hypotonia. Reduced muscle strength and endurance was also observed in an independent cohort on sensorimotor tasks during adulthood. Gait was assessed from postnatal day (P)21-30 and again at P60 using the DigiGait system for gait analysis. Our data reveal MYT1L reduces muscle strength and endurance during motor development likely resulting in hypertonia. Together, our findings will help us understand how this transcription factor influences the development of motor circuits. Funding from The Jakob Gene Fund, the Mallinckrodt Institute of Radiology at Washington University School of Medicine, McDonnell International Scholars Academy, and the NIH: (R01MH107515, R01MH124808 to JDD, and NIH 5UL1TR002345 (ICTS) and P50 HD103525 (IDDRC)).

Behavioural phenotyping of a genetic rat model of the Brain-Derived Neurotrophic Factor val66met polymorphism reveals selective impairment of fear memory.

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The val66met polymorphism is a common variant of the Brain-Derived Neurotrophin (BDNF) gene (rs6265) which reduces activity-dependent release of this neurotrophin and has been suggested as a risk factor for affective and psychotic disorders. Previous studies using transgenic val66met mice have shown impaired memory in both fear-based and spatial memory tasks. Here we present a behavioural characterization of a novel rat model of the BDNF val66met polymorphism. Methods: The knock-in rat line was originally established on a Sprague-Dawley genetic background using CRISPR/Cas-9 induced valine to methionine substitution at position 68, equivalent to position 66 in humans. Male and female rats of all three genotypes were used in a battery of behavioural tests to investigate anxiety-like behaviours and cognition. Results: Growth and development of the rats was normal. In a cued fear conditioning task, all genotypes showed similar fear acquisition and extinction learning. However, both male and female met/met rats (n=51 combined) showed a deficit in fear memory, expressed as a significant 26% and 19% reduction of freezing to the tone compared to val/val (n=45) and val/met rats (n=57), respectively. In contrast to fear memory, there were no genotype differences in Y-maze spatial memory or novel object recognition. There were also no differences in locomotor activity or anxiety-like behaviour in either the open field or elevated plus maze. ELISA showed similar BDNF levels in the ventral hippocampus between the genotypes, consistent with the polymorphism resulting in reduced BDNF release rather than resting levels. Conclusions: The val66met polymorphism in rats induced significant impairment of fear memory, but not other forms of memory or anxiety-like behaviour. These baseline data characterise this novel BDNF val66met rat model and warrant gene-environment studies to assess differential vulnerability between the genotypes in response to stress and drugs of abuse. Such studies are currently underway and preliminary results will be presented. Acknowledgement: The authors are grateful to Drs Caryl Sortwell and Timothy Collier (Michigan State University, Grand Rapids, MI, USA) for making the val68met rat line available to us.

Maternal Western-Style Diet offspring exhibit decreased social engagement and increased hippocampal volume during the periweaning period.

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Obesity is a preventable public health concern facing our society. Oxidative stress induced by obesity and the overconsumption of obesogenic diets, such as the Western-Style Diet (WSD), increases systemic inflammation (cytokine bioavailability) in the body. During pregnancy, this increased inflammatory profile can be reflected in the placenta and alterations to the placental immune milieu are associated with differences in offspring neuropsychiatric outcomes. Epidemiological studies have linked increases in Body Mass Index (BMI) during pregnancy with increased likelihood that offspring will be diagnosed with Attention Deficit Hyperactivity Disorder (ADHD) or Autism Spectrum Disorder (ASD). Using an established non-human primate model (macaca fuscatta) to investigate the relationship between maternal western-style diet (mWSD) and offspring behavioral outcomes, macaques were fed either a high-fat diet that calorically resembles the WSD or control diet through pregnancy and weaning. The behavioral phenotypes of offspring were assessed using the Novel Peer Introduction (NPI) task at six months of age. Results show that mWSD offspring display decreased prosocial behaviors (iniated proximity to partner (p = .02); affiliative contact (p = .05)) and increased repetitive behaviors (repetitive behavior composite, p=.03). Furthermore, MRI results show hippocampal volume may drive these behavioral observations, where mWSD offspring exhibit increased hippocampal volumes at four (p=.002) and six (p=.00015) months of age. Both the behavioral and volumetric results suggest mWSD macaque offspring display phenotypes similar to those described in humans diagnosed with an ASD. These findings support previous results that mWSD impacts fetal neurodevelopment and offspring postnatal behavior in ways that may increase likelihood of neuropsychiatric diagnosis.

Global Cerebral Ischemia in Male Long Evans Rats Does Not Increase Impulsive Choice Despite Significant Changes to Mesocorticolimbic Pathway Signalling

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Global cerebral ischemia (GCI), stemming from cardiac arrest, leads to disruptions of numerous behaviours and can result in behavioural impulsivity. As a subtype of impulsivity, impulsive choice can be quantified using delay discounting (DD) paradigms, which present a choice between a smaller reward delivered immediately (smaller, sooner) and a larger reward delivered at an increasingly long delay (larger, later). Moreover, impulsivity is largely regulated by the actions of dopamine (DA), notably within the DAergic mesocorticolimbic pathway. This study aimed at investigating the effects of GCI on DD performance while also quantifying changes in the mesocorticolimbic pathway. Eighteen male Long Evans rats, aged 21 days old at arrival, underwent 9 days of autoshaping training and 24 days of DD training prior to being subjected to GCI (n=9) or sham surgery (n=9). After recovery, rats completed behavioural testing followed by 6 days of postoperative DD sessions. Immunofluorescence was used to measure expression of DA receptor D2 (DRD2), DA transporters (DAT), and ΔFosB in the basolateral amygdala (BLA), nucleus accumbens core (NAcC) and shell (NAcS), and ventromedial prefrontal cortex (vmPFC). Analyses revealed that GCI did not lead to higher DD rates or number of omissions, but significantly decreased DRD2 expression in the NAcS, DAT immunoreactivity in the NAcC and NAcS, and ΔFosB expression in the NAcC with a trend towards a decrease in the vmPFC. Together, these results indicate that GCI yielded no impact on impulsive choice, despite significant alterations to the dopaminergic mesocorticolimbic pathway. This work was supported by a Discovery Grant from the Natural Science and Engineering Research Council (NSERC) of Canada appointed to HP [grant number RG203596-13].

Adolescent Stress Confers Resilience to Traumatic Stress in Adulthood: Role of the Prefrontal Cortex

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Stress during adolescence is usually associated with psychopathology later in life. However, under certain circumstances, developmental stress can promote an adaptive phenotype, allowing individuals to cope better with adverse situations in adulthood, thereby contributing to resilience. The aim of the study was to understand if and how adolescent stress may alter behavioral and physiological responses to traumatic stress in adulthood. Sprague Dawley rats were subjected to adolescent chronic variable stress (adol CVS) followed by single prolonged stress (SPS) in adulthood. After a week, animals were evaluated in an auditory-cued fear conditioning paradigm and neuronal recruitment during reinstatement was assessed by Fos expression. Patch clamp electrophysiology was performed to examine physiological changes associated with resilience. We observed that adol CVS blocked fear potentiation evoked by SPS. SPS impaired extinction (males) and enhanced reinstatement (both sexes) of the conditioned freezing response. Prior adol CVS prevented both effects. SPS effects were associated with a reduction of infralimbic (IL) cortex neuronal recruitment after reinstatement in males and increased engagement of the central amygdala in females, both also prevented by adol CVS, suggesting different neurocircuits involved in generating resilience between sexes. We explored the mechanism behind reduced IL recruitment by studying the intrinsic excitability of IL pyramidal neurons. SPS reduced excitability of IL neurons and prior adol CVS prevented this effect. Our data indicate that adolescent stress can impart resilience to the effects of traumatic stress on neuroplasticity and behavior by modulation of prefrontal (IL) excitability.This project was funded by the National Institutes of Health (R01MH101729, R01 MH049698 and R01 MH119814 to JPH, T32 DK059803 to EMC and SEM, F31MH123041 to NN).

Behavioral analysis of a Williams Syndrome mouse model to evaluate genetic contributions to sociability and anxiety.

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Williams Syndrome is a genetic, multisystemic, neurodevelopmental disorder which includes features of hypersociability, generalized anxiety, specific phobias, and learning disabilities. The underlying mechanisms of these symptoms are not well understood, but the genetic landscape of the disorder provides a unique opportunity to study them. Williams Syndrome is caused by a deletion of 26-28 genes on human chromosome 7, and duplication of this region produces opposite social features, suggesting a gene copy number effect. As a rare disorder, there is insufficient human data to fully research the link between these genes and the distinctive behavioral phenotypes resulting from their disruption, thus animal models are essential. Fortunately, the Williams Syndrome Critical Region is conserved in mice and a heterozygous Complete Deletion mouse model mirrors the human deletion. This study deeply phenotypes behaviors of the Complete Deletion mouse model while simultaneously investigating the potential copy number effects of a single gene of interest in the region, Gtf2ird1, which is suspected to contribute to hypersociability. Complete Deletion (CD) mice were bred to transgenic mice overexpressing Gtf2ird1 (OE). At least 20 mice of each genotype (WT, CD, OE, and CD/OE), were used to assess social and anxiety behaviors, in addition to other relevant traits. Social behaviors were measured with Social Approach and Social Operant paradigms. Anxiety was evaluated with Open Field, Marble Burying, and Elevated Plus Maze tasks. Conditioned Fear and Novelty Avoidance tasks were used to assess fear learning and reaction to novelty, respectively. Complete Deletion mice show deficits in many tasks (e.g., decreased time spent in the center during Open Field) that are not attenuated with Gtf2ird1 overexpression. A second, independent cohort will be assessed using additional tasks. The comprehensive results of this study will determine whether the Complete Deletion model is a useful tool for investigating the underlying mechanisms influencing social, anxiety, and fear behaviors in Williams Syndrome. Funding by NSF-DGE-1745038 (KRN) and 5R01MH107515-05 (JDD).

Estrogen and oxytocin receptors interact to mediate social recognition.

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Estrogens and oxytocin (OT) have been found to affect social behaviours, such as social recognition (SR). Various studies have found that 17b-estradiol (E2), estrogen receptor (ER) agonists, or oxytocin administered systemically or into various brain regions can facilitate SR. Conversely, knocking out the genes for the ERs, OT, or the OT receptor (OTR), or administering an OTR antagonist (OTRA) can impair SR. The findings that both estrogens and OT are needed for SR suggest they could be interacting. We have previously shown such interaction by finding that E2 infused into the paraventricular nucleus of the hypothalamus (PVN), one region where the majority of OT is produced in the brain, can rapidly facilitate SR and that this facilitation can be blocked by also infusing an OTRA into the medial amygdala, a region that has been found to be particularly important for OT-mediated SR. The current experiments aim to determine which ER in the PVN is mediating the interaction with OT. Since the G-protein coupled ER (GPER) and ER beta (ERb) are highly expressed in the PVN one or both receptors may be mediating this effect. To test this, specific agonists for these receptors (G1 for GPER; DPN for ERb) were infused into the PVN. We found that both agonists were able to rapidly facilitate SR. The current experiment is determining whether the same OTRA previously used can block the facilitative effect of either ER agonist. This is tested using a â€œdifficultâ€• SR paradigm, that was designed such that the vehicle group would not show SR and therefore it can be used to demonstrate facilitative effects of treatments. This paradigm involves two 5-minute sample phases, in which two stimulus mice are presented to the experimental mouse, followed by a test phase where two stimulus mice are presented again, however one is replaced with a novel mouse. Since mice have a preference to investigate novelty, enhanced investigation of the novel mouse suggests they recognize the previously encountered stimulus mouse. Our preliminary results show that the OTRA can block the facilitative effect of the GPER agonist on SR, but not the facilitative effect of the ERb agonist. This suggests that GPER, not ERb, in the PVN is mediating the interaction with the OTR in the medial amygdala to rapidly facilitate SR. Funded by NSERC.

Microstructural analysis of feeding behavior in young offspring of Wistar rats exposed to a sucrose-sweetened beverage during the peripregnancy period.

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In Mexico, according to the National survey of health and nutrition (ENSANUT, 2018) 85.8% of adults drinks Sugar-sweetened beverages (SSB). These are related to multiple metabolic disorders. SSB's intake in peripregnancy period increases the BMI in progeny, but the mechanisms undergoing this are not well understood. The aim of this work was to evaluate the microstructural analysis of feeding behavior (MAFB) of the young offspring of Wistar rats exposed to an SSB during peripregnancy period. We used 24 Wistar rats (12 controls and 12 from mothers exposed to an SSB [10% sucrose W/V] two hours per day [between 0900 and 1100 hours] during peripregnancy period [three weeks prepregnancy, pregnancy and lactation]). Weight and height were registered weekly from PD4 to evaluation day. At PD42, animals were video recorded during the first hour of dark cycle; Food and water intake were registered. MAFB were evaluated with eight parameters: Latency, duration and time between eating episodes; latency, duration and number of drinking episodes; time of activity and inactivity. At the end, animals were euthanized, and brain weight registered. Dams consumed between 10-20% of their daily calories from SSB. Experimental group were heavier and taller during the first four weeks of life. At PD42, not differences in body weight, food or water intake were found. However, changes in MAFB and brain weight were identified. In conclusion, SSB during peripregnancy period could alter body weight during earlier stages of life. Under a normal diet, these differences disappear before adolescences. By the way, differences in the MAFB could indicate that in the presences of alterations of the diet (a diet rich in fat or sucrose) the intake could easily increase, and elevate the body weight. However, more studies need to be done in order to understand the implications of the alterations of the feeding behavior in the body weight regulation.

Effect of the cued water maze training in glucocorticoid levels and long term potentiation related genes, SGK-1, BDNF and CK-2 in the rat dorsal striatum.

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Increased corticosterone release converges with stronger memory in aversive tasks performance, and this is related to the glucocorticoid receptor activity. Rats trained in the cued version of the water maze have shown that lesions in the dorsal striatum impairs memory, interestingly, the injection of corticosterone in this structure improve memory, this suggest that the dorsal striatum is involved in memory modulated by glucocorticoids. We evaluated the effect of the blockade of corticosterone synthesis by metyrapone administration in the consolidation of the cued water maze task, in the corticosterone plasma and striatum homogenate levels by ELISA and the glucocorticoid receptor phosphorylated in serine 211 (pGR-S211) by Western blot, as well as the mRNA expression by qPCR of SGK1, BDNF and CK2, that are associated with long-term potentiation mechanisms. We found an impairment in the retention test, which relates with a partial decrease in corticosterone for both plasm and dorsal striatum homogenate, however, there was no difference in levels of pGR-S211, the expression of SGK-1 and CK-2 increased in trained rats with or without the treatment of metyrapone. In summary, this work provides an early insight of the possible mechanisms of aversive memories in the dorsal striatum, in which the expression of SGK-1 and CK-2 genes could be involved. On the other hand, the impairment of the task performance in the subjects treated with metyrapone could be associated with the partial decrease in corticosterone levels of the dorsal striatum, raising the possibility of a modulatory effect, which could be independent of the transcriptional activity of pGR-S211. We acknowledge the technical assistance of Norma Serafin, Martha Carranza, Adriana GonzÃ¡lez, Andrea C. Medina, MartÃ-n GarcÃ-a, RamÃ³n MartÃ-nez, and Nuri Aranda. Supported by PAPIIT-UNAM (IN204118 and IN209621) and CONACYT (CB251634, Scholarship to RP-M CVU 700765).

Gait analysis across time in mouse and rat models of Angelman Syndrome

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Angelman Syndrome (AS) is a genetic neurodevelopmental disorder characterized by developmental delay, lack of speech, seizures, intellectual disability, and walking and balance disorders. We have focused on motor ability as an outcome measure as movement disorders including uncoordinated limb movement, delayed and abnormal walking and postural movements affect nearly every individual with AS. Motor outcomes present a strong opportunity for direct translational studies to investigate pharmacological, dietary, and genetic therapies. Clear phenotypes have been widely published on these phenotypes clinically (Wheeler et al., 2017) and preclinically (Born et al., 2017; Berg et al., 2020). One pilot study in children with AS showed abnormal and stunted walking compared to an age matched typical developing group (Grieco et al. 2018). To date, gait studies have not been reported in any preclinical model of AS. To fill this gap, we investigated temporal and spatial gait parameters in wild-type (WT) and AS mice and rats at alike juvenile and adult time points utilizing the DigiGait treadmill system with same parameters across species. In the mouse model, we observed robust deficits such as a wide unstable stance, increased stride length, and altered stride dynamics, compared to WT controls, at both the juvenile and adult time points. As rats showed a more subtle phenotype in the compared metrics, suggesting stronger compensation for any motor deficits in rats. Moreover, phenotype was more pronounced at the juvenile time point and was not detected at adulthood. Both species showed corroborating phenotypes in the gold-standard assays of open field and the accelerating rotarod. Our data highlights differing strengths of each species: mice are easier to conduct high throughout put gait analysis longitudinally and obtain a clear, reliable, easily measured output whereas rats do not show extreme motor phenotypes so motor will not confound social or cognitive behaviors as in the mouse. Future experiments will look at neonatal milestones to investigate earlier onsets of low motor ability. This is a critical discovery for aligning species and reliable, truly translational outcome measures for the numerous therapeutic approaches being tested for AS.Funding: FAST Foundation

Valproic acid (VPA) model rats have altered cerebellar volumes that may impact set-shifting performance.

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder with deficits in social interactions and executive function impairments. Our prior work has demonstrated that VPA treated offspring had impaired performance on an attentional set-shifting task. The current study used MRI and regions of interest analyses to measure the volumes of cerebellar subregions in VPA and controls rats that had participated in the attentional set-shifting task. VPA males had significantly more volume in lobule VI compared to male controls. VPA female rats had significantly less volume in lobules I, IV and X compared to female controls. In addition, decreases in volume for VPA females was associated with worse performance. Males with increases in lobule VI were also impaired on the set-shifting task. Similar volumetric differences within the cerebellum have been observed in humans with ASD, which suggests that the VPA model is capturing some of the same brain changes observed in humans with ASD, and that these changes in volume may impact cognition.This work was funded by a pilot grant awarded to Dr. Plakke from CNAP supported by National Institute of General Medical Science GM113109 NIH, start up funds and a USRG from KSU to Dr. Plakke.

Maternal age and 17b-estradiol influence cognition in middle-aged rats.

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Female-specific characteristics, such as pregnancy, can influence disease risk. Estrogens (primarily estrone and estradiol) can improve cognition in postmenopausal women and women with AD, but their effects vary dramatically across studies. This is in part due to different compositions and doses of estrogens. However, other factors such as pregnancy and motherhood (parity), can have long-term effects on cognition and brain plasticity in both humans and rodents. Past research from our lab indicates that previous parity influences the neuroplastic ability of the hippocampus to respond to estrogens in middle age. Acute estrogens increase cell proliferation in the hippocampus in middle age in multiparous, but not in nulliparous rats. In the present study, we examined whether maternal age (age of first pregnancy) and 17b-estradiol treatment differentially affected hippocampal neurogenesis and cognition in middle-age. Female rats were either bred at 3 months, 6 months, or were nulliparous (never bred). At 13 months, rats received daily injections of 0.03ug 17b-estradiol (or sesame oil vehicle) for sixteen days. From day 12-16, rats were trained on the standard reference memory version of the Morris water maze, following which they performed a probe trial and reversal training paradigm. Our findings indicate that younger maternal age coupled with 17b-estradiol was associated with impaired reference memory performance compared to controls. However, advanced maternal age showed greater cognitive flexibility, regardless of 17b-estradiol treatment. Our findings suggest that maternal age and 17b-estradiol can influence cognitive performance in middle age.Funding by CIHR (PJT-148662)

Maternal postpartum corticosterone and fluoxetine treatment has long-term effects on offspring hippocampal neurogenesis and neuroinflammation.

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Perinatal depression (PND) affects 15% of mothers and selective serotonin reuptake inhibitors (SSRIs) are the first-line treatment for PND. However, SSRI efficacy for mood alleviation in mothers and safety to infants have been questioned. We previously reported that maternal SSRI exposure in the postpartum period increased hippocampal IL-1Î² levels, which may be tied to the limited efficacy of SSRIs in the late postpartum period. However, it is not yet known whether maternal postpartum SSRIs affect the neuroinflammatory profile of adult offspring. We hypothesize that maternal corticosterone (CORT) treatment used to induce depressive-like endophenotypes in dams, and postpartum SSRI treatment will alter offspring puberty development, neurogenesis, and neuroinflammation in adulthood. CORT (40mg/kg, s.c) was given to dams in the postpartum to model de novo postpartum depression, with or without the SSRI fluoxetine (FLX; 10mg/kg, s.c.), for 21 days. We found maternal FLX treatment decreased hippocampal IFN-Î³, IL-10, and IL-13, whereas maternal CORT increased hippocampal IL-13 in both sexes. Principal component analysis revealed that maternal FLX treatment decreased the overall neuroinflammatory profile. Maternal FLX treatment reduced adult hippocampal neurogenesis in the ventral dentate gyrus in both sexes. Moreover, maternal CORT treatment delayed puberty development in female offspring only. These data underscore that postpartum depression and SSRI treatment in dams can have long-term effects on offspring, some of which are dependent on sex. This work was funded by a Canadian Institutes of Health Research (CIHR) operating grant (MOP142308) to LAMG.

Repeated morphine administration alters prelimbic cortex activity and increases risk-taking behavior during an approach-avoidance conflict task.

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Opioid addiction is characterized by risky drug use. However, how the brain processes risk in the context of opioid use is unclear. We hypothesized that repeated opioid exposure alters prefrontal cortex activity, leading to increased risk-taking behavior. To test this hypothesis, we designed an approach-avoidance conflict model that involves the presentation of a fear-inducing predator odor after opioid place preference . We injected adult male Long-Evans rats with saline (n=28) or morphine (n=21) and exposed them to the least-preferred side of the chamber. Morphine and saline conditioning occurred on alternating days over a 10-day period. After 72 h of forced abstinence, morphine-treated rats showed a preference for the drug-paired side ($p=0.008$). Immediately after preference testing, we added predator odor (cat saliva) to the previously drug-paired side to create a motivational conflict. While the saline group showed aversion to cat odor during the conflict test ($p<0.001$), the morphine group continued to enter the drug-paired side ($p=0.449$), demonstrating that morphine conditioning induces an increase in risk-taking behavior. Single-unit recordings from PL neurons revealed a significant number of cells with suppressed firing rates after acute administration of morphine (22%, 19/86), but not saline (6%, 5/64; $p=0.011$). On the last conditioning day, morphine failed to suppress PL neuronal firing rates as compared to saline ($p=0.397$), suggesting that PL neurons adapt to the effects of morphine over time. During the conflict test, many PL cells showed increased spontaneous firing rates when saline group animals entered the cat odor-paired side (39%, 11/28), though this broad excitation was not seen in morphine group animals during risk taking (17%, 10/59; $p=0.032$). Taken together, our results demonstrate that repeated opioid exposure elicits the expression of reward-related memory, as well as physiological adaptation of PL neurons to the effects of opioids. In addition, following repeated opioid exposure, PL neurons show decreased spontaneous excitatory tone that correlates with increased risk-taking behavior during motivational conflict.

Longitudinal development of cortical calcium functional connectivity in the healthy mouse and a mouse model of Rett Syndrome.

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Individual differences in the developmental trajectories of the disordered brain drive a need to understand longitudinal neurodevelopment in the healthy mouse. Characterization of functional connectivity development in the healthy mouse and a mouse model of Rett Syndrome can provide essential insight into how behavior and other symptoms progress in neurodevelopmental disorders. To this end, we collected resting-state calcium functional connectivity (FC) data using an optical fluorescence imaging system in both the healthy developing mouse and in the Mecp2 mouse model of Rett Syndrome. Data were acquired to characterize FC development across 5 longitudinal timepoints (postnatal day 15 (P15), P22, P28, P35 and P60) in 17 healthy C57Bl/6J mice expressing the genetically encoded calcium indicator GCaMP6f directly under the Thy1 promoter. We observed the largest change in functional connection strength and a rebalancing of intra- and interhemispheric connections between P15 and P22 in healthy mice, suggesting that the period of most dynamic developmental FC change occurs before 4 weeks of age. Both sensorimotor and association cortices had developmental trajectories which displayed significant change in FC between P15 and P60, with multiple regions displaying an increase between P15 and P22 and then a subsequent decrease by P60. We also collected resting-state calcium FC data during development (P35) and in adulthood in the Mecp2 mouse model of Rett Syndrome, both with and without a cell-specific rescue-of-function in GABAergic neurons. We then evaluated the correlation between FC measures and their symptom profile, as measured by Bird score and Rotarod metrics. Preliminary analysis suggested substantial changes in FC during development in the Mecp2 model. This examination of healthy brain development as well as perturbations in the Mecp2 mouse provides new insight into how functional brain networks may be altered and correspond to age and disease state in Rett Syndrome and other neurodevelopmental disorders.Funding Acknowledgement: NINDS F31NS110222

Quiescent wakefulness: The influence of oxytocin on sleep-wake behaviour and modulatory role of sex and route of administration.

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Oxytocin is a hypothalamic neuropeptide that exerts diverse physiological and neurobehavioural effects. Based on its stress-suppressant and serenic effects, one could hypothesise that oxytocin should elicit sleep-promoting effects. However, the social salience hypothesis suggests that oxytocin promotes prosocial behaviour and directs attention toward social stimuli, so one could also posit that oxytocin should promote wakefulness. At present, little research has comprehensively characterised the influence of oxytocin on sleep-wake behaviour and no explanation to reconcile these two seemingly competing hypotheses has been proposed. This study investigated the effects of oxytocin on sleep-wake outcomes using radiotelemetry-based polysomnography in adult male and female Wistar rats. Oxytocin was administered via the intraperitoneal (IP; 0.1, 0.3 and 1 mg/kg) and intranasal (IN; 0.06, 1, 3 mg/kg) routes. Caffeine (IP and IN; 10 mg/kg) was also administered as a wake-promoting positive control. Additionally, pre-treatment with the oxytocin receptor (OTR) antagonist L-368,899 (IP; 5 mg/kg) and vasopressin 1a receptor (V1aR) antagonist SR49059 (IP; 1 mg/kg) followed by oxytocin (IP; 1 mg/kg) was conducted to determine which receptor(s) mediated sleep-wake effects of oxytocin. In both male and female rats, IP oxytocin produced dose-dependent effects on sleep-wake behaviour. Specifically, oxytocin initially promoted quiescent wakefulness at the cost of reducing active wakefulness, NREM and REM sleep. Conversely, IN oxytocin did not significantly alter most sleep-wake parameters at any dose tested. Caffeine demonstrated wake-promoting effects under both the IP and IN routes of administration. The involvement of OTR and V1aR binding in oxytocin-induced effects on sleep-wake outcomes will be discussed. These findings appear to reconcile the two competing hypotheses: in rats, IP oxytocin appears to promote a state of "quiescent wakefulness": one of calm and rest, but also of conscious responsivity to environmental stimuli.

Temporal control of hunger-sensing AgRP neurons is critical for context-conditioned overeating in mice.

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Context exerts powerful control over feeding behaviour through learned associations. Rodents can be trained to over-eat in response to a specific context, but only if training occurs under calorie-restricted conditions. This suggests hunger circuits play a key role in development of the conditioned association. Peripheral hunger signals are detected by hypothalamic Agouti-related peptide (AgRP) neurons that promote feeding and food-seeking. It is currently unknown whether AgRP neurons present a link between hunger states and context-conditioned feeding. To test this, we trained AgRP-cre (n=18) and WT littermates (n=25) to associate consumption of a palatable food with a specific context (context A; food-paired context) for 30 minutes/day while either hungry (fasted or AgRP-activated) or full (fed or AgRP-inhibited). A distinctly different context without food (context B; unpaired context) served as a control. Mice were later probed for a context-conditioned feeding response by comparing food intake in A vs B; an effect absent in fed controls. We first showed that AgRP neurons are necessary for the fasting-mediated effect, by blocking AgRP activity (HM4Di) in fasted mice prior to training. CNO (3mg/kg) 60 minutes prior to training sessions prevented a later context-conditioned response (p< 0.05). We therefore asked whether AgRP activation alone is sufficient to form a context-conditioned feeding response in fed mice, using two approaches: (i) activating AgRP-expressing Gq DREADDs (HM3Dq) with CNO (1mg/kg) prior to training, and (ii) stimulating AgRP neurons for 20 minutes selectively within the training context, using a soma-targeted optogenetic approach (SoCoChR; 10mW blue light). Both approaches similarly produced spontaneous feeding during training (p< 0.05), however; only the context-specific optogenetic approach translated to a context-conditioned response at test (p< 0.05). Therefore, we suggest fasting mediates context-conditioned feeding through AgRP neurons, but that temporally controlled inactivation may be critical for conditioned feeding.These findings offer insight into the fundamental processes governing conditioned appetite and non-homeostatic feeding that underscore eating disorders and obesity.

The integration of energy homeostasis with food reward requires glucose-sensing in Agrp neurons.

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AgRP neurons increase food motivation by engaging dopaminergic rewards circuits but how these neurons sense hunger to influence dopamine release and motivated behaviour has not been addressed. We hypothesized that metabolic sensing of calorie availability is necessary for AgRP neurons to transmit energy-state information to dopaminergic reward pathways. To assess the role of metabolic sensing in AgRP neurons and the effects on reward and motivation, we confirmed that deleting the metabolic enzyme carnitine acetyltransferase (Crat) impaired glucose-sensing in AgRP neurons using electrophysiology. Two-bottle choice tests show that Crat in AgRP neurons is important for sensing of the caloric value of sweet solutions since fasting increases sucrose consumption in WT more than in KO mice. Moreover, during fasting WT mice will still consume sucrose spiked with quinine (unpleasant tastant) to consume calories as required, whereas KO mice do not. Since the dorsal striatum mediates the rewarding properties of caloric but not taste information, we developed a whole animal PET/CT f18DOPA scan method to estimate dopamine activity of the dorsal and ventral striatum system. Our results showed lower f18DOPA uptake in the dorsal striatum of KO mice in response to reward stimulus compared to WT mice 1 hour after palatable food consumption. To assess acute dopamine dynamics in awake, free behaving mice, we employed GRAB dopamine sensors and recorded dopamine release in dorsal striatum and nucleus accumbens using fibre photometry during feeding and operant conditioning tasks. We show that the absence of Crat in AgRP neurons reduced motivated behaviour and dopamine release in the nucleus accumbens during operant reward-seeking and palatable food consumption. These studies demonstrate that appropriate glucose-sensing via Crat in AgRP neurons is required to detect and translate low energy states into increased dopamine release in reward pathways and increased motivated responding for food rewards. The studies highlight that hunger-sensing in AgRP neurons potentiate dopamine release and specifically identify Crat in AgRP neurons as a potential molecular target to reduce motivation to consume food rewards. Acknowledgement: AR was supported through a Postdoctoral bridging fellowship and Platform access grant from Monash University

Basolateral amygdala corticotropin-releasing factor circuitry recruited during cocaine-memory reconsolidation.

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Drug-paired memories become labile upon retrieval; thus, their long-term maintenance requires their reconsolidation into long-term memory stores. Drug-memory reconsolidation requires signaling through corticotropin-releasing factor receptor type 1 (CRFR1) in the basolateral amygdala (BLA). However, the source of critical CRF release in the BLA during memory reconsolidation has not been determined. To address this gap in knowledge, we utilized retrograde tracing, behavioral manipulations, and multi-label immunohistochemistry to identify BLA-projecting CRF neuronal populations that exhibit an increase in neuronal activation during cocaine-memory reconsolidation. Male and female Sprague-Dawley rats received bilateral RetrobeadTM infusions into the BLA and an indwelling catheter into the jugular vein. They then received training to lever press for cocaine infusions in a distinct context, followed by extinction training in a different context. Rats were then exposed to the cocaine-paired context for 15 minutes to reactivate context-cocaine memories and initiate reconsolidation or were left in their home cages (controls). Brain tissue was collected 2 hours later to assess c-Fos expression, an index of neuronal activation, ~30 minutes into reconsolidation. RetrobeadTM labeling as well as CRF and c-Fos immunoreactivity were visualized in serially sectioned brain tissue using standard confocal microscopy protocols. Single-, double-, and triple-labeled cells were quantified by two independent observers blind to group assignment. BLA-projecting CRF-immunoreactive neurons in the anterior cingulate cortex (ACC), prelimbic prefrontal cortex (PrL), and dorsal raphe (DR) exhibited increased c-Fos expression following cocaine-memory reactivation (during reconsolidation) relative to home cage controls. These cell populations also expressed CAMKII, but not GAD67, immunoreactivity, suggesting they co-release glutamate. These data suggest that the PRL, AC, and/or DR may send requisite CRF input to the BLA during memory reconsolidation and provide impetus for further investigation into the role of these CRF circuits in cocaine-memory reconsolidation. Funding for this research was provided by NIH NIDA 2 R01 DA025646 and by Washington State Initiative 171.

Pharmacological and contextual modulation of grooming syntaxes and ultrasonic vocalizations

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In rats, the emission of grooming in unconditioned anxiety tests such as the open field (OF) is considered as an indicator of negative emotionality. However, new evidence suggests that complex grooming forms act as a compensatory mechanism of emotional de-arousal, while other short sequences directed to the head and forepaws resemble an ongoing stress states. We recently demonstrated that an acute stress experience (i.e., foot-shock) prior to an OF assessment reduce the overall emission of grooming while increases cephalic sequences. Such experience caused no changes on exploratory and risk-assessment behaviors (E&RA), but cause a strong increasing of 22-KHz distress calls. To expand the evidence about the association between grooming sequences and stress, we tested how the administration of the endogenous stress hormone, corticosterone (CORT), altered grooming syntax in the OF after an acute stress experience. For that purpose, animals were ip injected with vehicle (VEH) or CORT (40mg/kg) 15min before being exposed to a shock chamber. After 1min of habituation, half of the animals received three foot shocks and then were assessed in an OF during 15min. We also aimed to study if a later exposure to the shock chamber in the absence of stressors was capable of alter animals' behavior in the OF. For that purpose, rats were re-exposed to the shock chamber 24h later, and then they were newly assessed in the OF. A group of naÃ¯ve animals were tested in two 24h-appart OF as controls of the behavioral alterations caused by the ip injection and the shock chamber exposure. Evidence will be shown about the putative effect of pharmacologically- and contextually induced stress on grooming behavior and 22-KHz distress calls, but also of its modulation on the traditional E&RA parameters. Here, we show evidence supporting the hypothesis of the emission of complex grooming sequences as a compensatory mechanism of emotional de-arousal. Finally, we propose that the detailed analysis of grooming over time provide a rich array of information useful for different preclinical models.Acknowledgement: This work was founded by the projects 837-B8-123, University of Costa Rica.

EEG spectral power changes in profoundly deaf and normal hearing individuals during tactile temporal discrimination.

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Challenges in early oral language acquisition in profoundly deaf individuals have an impact on cognitive neurodevelopment. This has led to the exploration of alternative sound perception methods involving training of vibrotactile discrimination of sounds within the language spectrum. In particular, stimulus duration plays an important role in linguistic categorical perception. We comparatively evaluated vibrotactile temporal discrimination of sound and how specific training can modify the underlying electrical brain activity. Fifteen profoundly deaf (PD) and 15 normal-hearing (NH) subjects performed a vibrotactile oddball task with simultaneous EEG recording, before and after a short training period (5 one-hour sessions; in 2.5-3 weeks). The stimuli consisted of 700 Hz pure-tones with different duration (target: long 500 ms; non-target: short 250 ms). The sound-wave stimuli were delivered by a small device worn on the right index finger. A similar behavioral training effect was observed in both groups showing significant improvement in sound-duration discrimination. However, quantitative EEG measurements reveal distinct neurophysiological patterns characterized by higher and more diffuse delta band magnitudes in the PD group, together with a generalized decrement in absolute power in both groups that might reflect a facilitating process associated with learning. Furthermore, training-related changes were found in the beta-band in NH. Findings suggest PD have different cognitive adaptive mechanisms which are not a mere amplification effect due to greater cortical excitability.

Inducible Calling Cards: Developing A Novel Mouse Reagent for Temporally Controlled Recording of Epigenetic States

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In many psychiatric disorder models, such as addiction, chronic stress, and depression, genetically identical rodents are classified as either â€œvulnerableâ€• or â€œresientâ€• based on distinct behavioral responses to the same environmental manipulation. This individual-level vulnerability is proposed to be caused by epigenetic state prior to the exposure. To link antecedent states to eventual classifications, the same animals need to be studied molecularly (before the exposure) and behaviorally (after it). Hence, there is an urgent need to â€œfreezeâ€• molecular states prior to behavioral manipulation. We previously developed Calling Cards (CC) to nondestructively and permanently record transient transcription factor (TF)-DNA interactions in the live mouse brain. CC consists of a hyperpiggyBac (hyPB) transposase which, when linked to a TF, inserts self-reporting transposons (SRTs) near the TF's genomic binding sites. However, current CC reagents continuously record once turned on, meaning behavioral manipulations would influence post-mortem CC readouts. Thus, we are developing inducible CC mouse lines to permit initiation and termination of recording of TF-DNA binding at will. Our test case TFs are SP1, which binds active promoters, and Jun, an immediate early gene. Using a tamoxifen (TAM) inducible CC mouse line and fluorescent reporters, we established that the TF-hyPB transposase activity is silent without induction and can be activated in a specified temporal window by TAM delivery. Furthermore, using SRTs coupled with high-throughput sequencing, we recovered tens of thousands of recorded TF binding events. Our promising results indicate inducible CC may uniquely allow retroactive analysis of an animal's epigenetic state prior to a manipulation that has alternative behavioral outcomes. We are continuing to benchmark these reagents and characterize the efficacy, general health and behavior of these new mouse lines. We believe inducible CC mouse reagents could prove valuable for behavioral genetics research broadly, on topics including but not limited to social development, early life adversity, and stress resilience.This work was supported by NIH Grants U01MH10913301, RF01MH117070-01, and R21HG009750 and the WUSM Hope Center Viral Vectors Core.

Cannabidiol alleviates opioid withdrawal but worsens the development of tolerance to the analgesic effects of opioids.

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The opioid epidemic is the deadliest drug crisis in American history, taking 130 lives each day, more than guns or car accidents. Prescription opioids usurp the brain's reward system while creating tolerance and withdrawal, gating the transition to using more dangerous opioids. Emerging evidence suggests a potential role of cannabis for opioid use disorder. For example, opioid use decreased by 64-75% in chronic pain populations using medicinal cannabis, and opioid prescriptions and mortality rates are lower in US states that have legalised medicinal cannabis. However, the current panacea-like view of cannabinoids is problematic given the lack of systematic pre-clinical or clinical studies exploring their efficacy. In this research, we specifically examined the use of cannabidiol (CBD) for the prevention of physical dependence to opioids. Specially, we explored the efficacy of chronic co-administration of CBD with oxycodone for the attenuation of opioid withdrawal and tolerance to the analgesic effects of opioids in mice. To elicit opioid withdrawal, mice received escalating doses of oxycodone twice daily for 9 days, then underwent a 24hour abstinence period followed by an examination of gastrointestinal, somatic, and negative affective symptoms of opioid withdrawal. To examine opioid tolerance we administered a consistent dose of oxycodone twice daily over 5 days, followed by tests of thermal pain sensation. CBD was administered prior to each oxycodone injection, excluding test day. We discovered that chronic co-administration of CBD alongside oxycodone dose dependently inhibited the emergence of opioid withdrawal-induced jumps, an escape behaviour thought to be caused by dysphoria experienced during withdrawal. Surprisingly, CBD co-administration accelerated the development of tolerance to the analgesic effects of oxycodone. Genetic studies are underway examining the effect of CBD on the expression of key receptors involved in the development of opioid tolerance.

Environment enrichment versus social grouping has differential effects on long-term behavioral deficits in a mouse model of pediatric traumatic brain injury.

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Social and cognitive impairments are common after traumatic brain injury (TBI) during childhood. Rehabilitation strategies may support behavioral recovery; however, such interventions have rarely been systematically evaluated for efficacy. Here, we asked whether the social environment can modulate long-term behavioral outcomes in young mice after experimental TBI, compared to typical environmental enrichment (EE), which incorporates sensory, motor and cognitive stimuli. Male C57Bl/6J mice received a moderate-severe TBI or sham surgery at postnatal day 21. After one week, mice were randomized to different social conditions (minimal socialization, n=2/cage; or social group, n=6/cage), and housing conditions (standard cage, or EE). Objects in EE cages were rotated twice-weekly for novelty. From 8 weeks, mice underwent a series of behavioral tests (open field, elevated plus maze, social interactions, scent marking, three-chamber social approach, rotarod, Y-maze, and Morris Water Maze). From preliminary analysis (n=5-9/group), TBI mice showed hyperactivity, spatial memory deficits, reduced anxiety-like behavior, and reduced sensorimotor function compared to age-matched sham controls, as well as reduced social/sociosexual behaviors. EE appeared to alter anxiety-like behavior in TBI mice, increase sensorimotor performance in both TBI and sham mice, and increase reciprocal social interactions. Social grouping appeared to reduce hyperactivity and alter anxiety-like behavior in TBI mice, but had little effect on social behavior. Surprisingly, cognitive outcomes were not influenced by EE or social grouping. Findings indicate that manipulating the post-injury environment yields beneficial effects on chronic behavioral outcomes, which may be specific to the type of enrichment (social or otherwise). Ultimately, this study will improve our understanding of modifiable factors that may be harnessed to promote optimal long-term outcomes for survivors of early life TBI. Funding: NHMRC (Australia).

Hunger sensing via the olfactory bulb affects behavior and metabolism.

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Eating behaviors, particularly overeating, are not only driven by nutritional requirements but also the sensory perception of food cues that predict a food reward. Olfaction is often the first sensory cue of food availability and olfaction influences mood, motivation, and memory. Individuals that can't smell show changes in the enjoyment of food and olfactory dysfunction is associated with mental and metabolic illnesses. Importantly, smell perception increases when hungry or is impaired with metabolic diseases, such as obesity. The mechanism linking metabolic state and olfaction remains unknown. Ghrelin is known as the â€œhunger hormoneâ€• and regulates metabolism, mood, and memory at various central nervous system (CNS) locations via its receptor under a state of energy deficit. Although the ghrelin receptors are highly expressed in the olfactory bulb (OB), their function remains unknown. We investigated whether ghrelin receptors in the OB affect olfaction and whether or not this influences mood and metabolic parameters using a viral genetic knockdown approach to chronically delete ghrelin receptors specifically in the OB in ghrelin receptor floxed mice. Deletion of ghrelin receptors in the OB significantly affected olfactory performance in olfactory discrimination and habituation tasks in both fed and fasted mice, as well as increased the latency to find food under both fasted and ghrelin-induced conditions. A two-bottle choice assay for saccharin vs water indicated that mice lacking ghrelin receptors in the OB were completely anhedonic and did not show a preference for saccharin. In support of this, we observed significantly increased anxiety and reduced exploratory locomotor activity in 3 independent anxiety behavioral tasks. Intriguingly, mice increased body weight, fat mass, and blood glucose, indicating metabolic dysfunction. We conclude that OB GHSR maintains olfactory sensitivity under fasted conditions, leading to a number of behavioral and metabolic adaptations to help a mammal detect and respond appropriately to food and odor cues. The study was supported by the Australian Research Council as well as the Australian National Health and Medical Research Council.

MicroPET evidence for a hypersensitive neuroinflammatory profile of a gp120 mouse model of HIV.

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Despite the major increases in survival rates for people living with HIV (PLWH), there remain cognitive and behavioral abnormalities that negatively impact their lives. It is therefore critical to determine the mechanisms underlying these abnormalities to alleviate this impact. Human positron emission tomography (PET) studies reveal that PLWH exhibit elevated levels of neuroinflammation, known to impact cognitive and behavioral function in numerous psychiatric conditions. Understanding the mechanisms driving this neuroinflammatory profile relevant to HIV is key for targeted therapeutics to be developed. Gp120 is the glycoprotein that enables virus entry into a cell contributing to HIV-related neurotoxicity and its overexpression enables delineating the impact of such insertion. Gp120 transgenic (Tg) mice exhibit several HIV-relevant aspects, e.g., in vitro neuroinflammatory markers GFAP, Iba-1, IL6, plus behavioral, neurophysiological deficits, including impaired reinforcement learning, executive functioning, risk-taking, and drug sensitivity. To-date however, confirmation of gp120-induced in vivo neuroinflammation in these mice has yet to be determined. Here, we conducted microPET imaging of neuroinflammation via TSPO quantification with 18F-FEPPA radiotracer binding both at baseline and 24-hours after treatment with the inflammatory agent lipopolysaccharide (LPS; 5 mg/kg) in male gp120 Tg and wildtype mice. We observed that gp120 Tg mice exhibited a hypersensitivity to LPS, relative to wildtype littermate mice. These data indicate that gp120 Tg mice are hypersensitive to environmental insult-induced neuroinflammation consistent with PLWH as they also exhibit heightened sensitivity to infectious agents. Future studies should determine whether this heightened sensitivity is connected to the behavioral abnormalities of these mice. Gp120 Tg mice remain a useful model for delineating the mechanisms contributing to heightened sensitivity to neuroinflammation seen in PLWH.Funding: Research reported was supported by NIDA of the National Institutes of Health under award number R01DA044909.

Effects of noradrenergic activation and blockade on contextual memory specificity in rats.

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Extensive evidence indicates that norepinephrine plays an important role in strengthening contextual memory consolidation. Although several studies have shown that noradrenergic activation can enhance memory strength, divergent results regarding its effects on memory specificity have been reported. The aim of this study was to investigate the effects of noradrenergic stimulation and blockade on contextual memory specificity, through systemic administration of the Î±2 adrenergic antagonist yohimbine and the ÃŸ adrenergic antagonist propranolol, respectively. Wistar male rats were submitted to the contextual fear conditioning (CFC) task. CFC training was performed in conditioning chambers where different groups of rats were presented with three foot-shocks (0.3 or 0.6mA, yohimbine-treated animals - 0.6 or 1mA, propranolol-treated animals) and immediately after training, received subcutaneous injection of yohimbine (0.3 or 1.0mg/kg), propranolol (10mg/kg) or vehicle (0.9% saline). Two days after training, a discriminative CFC test was performed placing each subject first in a novel context (B) and then to the training context (A), in this order. The freezing time was assessed in both contexts as a measure of memory strength and specificity. Vehicle-treated animals trained with the 0.3mA shock showed equivalent freezing levels through both training and novel contexts, which indicates non-specific fear conditioning and, thus, a generalized memory. Yohimbine-treated animals trained with the 0.3mA shock showed higher freezing levels when tested in context A, compared to context B, on both drug doses. Animals trained with the 0.6mA shock showed higher freezing levels in context A when compared to context B, independent of the treatment, as well as animals trained with the 1.0mA shock. These results suggest that noradrenergic activation enhanced memory specificity at recent time-point and weak training intensity, but has no effect on animals who received a mild training intensity. The noradrenergic blockade by propranolol showed no effect on both training intensities. Our results suggest that the noradrenergic system plays a complex and dynamic modulatory role in memory specificity, depending mainly on training intensity.

Anxiety and depressive symptoms in humans are related to distinct avoidance profiles in a cross-species translation of a go/no-go avoidance task.

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Anxiety and depression show notable heterogeneity in symptomology. Traditional classification methods have failed to reliably predict treatment efficacy, suggesting incomplete understanding of underlying pathophysiologies. The Research Domain Criterion (RDoC) initiative seeks to improve classification by strengthening the translational link between animal models and psychiatric disorders. Impaired avoidance behaviors are central features in both anxiety and depression but human measures of avoidance are typically self-reported and, unlike rodent models, do not discriminate active from inhibitory avoidance. Therefore, developing a translational task that is sensitive to differences in active vs. inhibitory avoidance in humans is critical. Undergraduates were assessed for anxiety and depressive symptoms using the Beck Anxiety Inventory (BAI) and the Beck Depression Inventory (BDI-II), respectively. To investigate active and inhibitory avoidance in humans, we created a three-stage, go/no-go avoidance task analogous to a rodent paradigm. The task used visual cues to signal the type of response (go/active vs. no-go/inhibitory) required to avoid an aversive noise. An acquisition stage involved learning an active avoidance rule. An intermixed stage required learning active and inhibitory avoidance responses. Finally, in a reversal stage, the response rules were reversed. Transitions between task stages were unsignalled. High anxiety participants were impaired in active avoidance, showing reduced accuracy on Go trials during all 3 task stages compared to low anxiety participants. There was no difference between high vs. low anxiety participants in inhibitory avoidance performance. High depressive participants were impaired in active avoidance during the acquisition stage but showed higher levels of active avoidance during the intermixed stage compared to low depressive participants. There was no difference between high vs. low depressive participants in inhibitory avoidance performance. This translational task may provide future utility in furthering our understanding of the pathophysiologies that underlie anxiety and depression.

Pathway-specific chemogenetic neuromodulation enhances working memory in rhesus monkeys.

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Acetylcholine plays a critical role in promoting neuronal plasticity and shaping synaptic connections throughout the brain, largely by projections from the basal forebrain cholinergic system. Although systemic pro-cholinergic drugs and electrical deep brain stimulation of the basal forebrain improve memory in nonhuman primates and humans, it has yet to be shown whether circuit-specific activation of a cholinergic neuromodulatory system can improve cognitive performance. Using a dual-viral intersectional approach, we transduced hM3D-Gq-coupled DREADDs (designer receptors exclusively activated by designer drugs) into the basal forebrain, to reversibly activate projections from the nucleus basalis of Meynert to the dorsolateral prefrontal cortex. We tested whether circuit activation could overcome deficits in a spatial-delayed response task caused by the muscarinic antagonist scopolamine (SCOP) or by presentation of a distractor in two young male rhesus monkeys. In the spatial-delayed response task, working memory performance was assessed after combined intramuscular injections of either SCOP and vehicle (VEH), the DREADD actuator deschloroclozapine (DCZ) and VEH, or a combination of SCOP and DCZ. In the distractor task, working memory was assessed following injection of VEH or DCZ. In the spatial-delayed response task, monkeys showed significant working memory improvement after SCOP plus DCZ injection compared to SCOP alone, indicating that the activation of the nucleus basalis to dorsolateral prefrontal circuit could offset working memory impairment caused by the cholinergic antagonist. In the distractor task after VEH, monkeys showed significant memory impairment after the distractor compared to no distractor. Notably, monkeys showed significant improvement in the distractor condition following DCZ injection compared to performance after VEH. These findings may provide a novel potential neurotherapeutic approach for circuit-specific treatment of cognitive impairments seen in aging and disease that result from deficits in cholinergic neuromodulation. This work was supported by R21NS096936 (MGB) and T32AG049688 (NAU).

Prenatal immune challenge causes behavioural and hippocampal proteomic changes at the synapse in adult mice, relevant for neuropsychiatric disorders.

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The purpose of this project was to further our understanding of the molecular changes following maternal immune activation (MIA) - a prenatal model of neurodevelopmental psychiatric disorders (NDDs). Thus far, multiple transcriptomic studies of the model exist but proteome-level investigations are lacking. We combined extensive behavioural phenotyping for the investigation of abnormalities in immune-challenged offspring of both sexes with a proteomic analysis of hippocampal (HPC) tissue. MIA was induced on gestational day 9 via a viral mimetic in pregnant C57Bl/6J dams. Exploration, anxiety- and depression-like behaviours, social interaction, and others were examined in treated offspring and controls (n=50). HPC synaptoneurosomes (n=4/group) were quantified by label-free LC-MS/MS using the data independent acquisition mass spectrometry method. Enrichment, network, and pathway analyses were performed for differentially expressed proteins. Multiple, partially sex-dependent, NDD-linked changes in behaviour, including depression-like traits, abnormal exploration and novelty response were observed in offspring of treated dams. In-depth analysis of HPC proteomic data revealed highly significant enrichment of pathways and networks involved in corresponding behaviours and psychological disorders. Top networks for observed changes included protein synthesis, nervous system development and disorders, as well as cell-to-cell signalling and interaction. Enriched canonical pathways of interest included multiple neuronal signalling pathways, involved in translation and synaptogenesis, and highlighted the glutamatergic synapse. Future studies will combine MIA with our genetic models to focus on gene x environment interactions and their molecular correlates. Funding: The project was supported by the German Research Foundation, the German Academic Exchange Service, the National Institutes of Health National Cancer Institute, the Academy of Finland, and the Polytechnic Foundation of Frankfurt am Main.

Behavioral effect of selective deletion of beta2* nicotinic acetylcholine receptors in neuropeptide Y expressing interneurons

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Neuropeptide Y (NPY) is an abundant neuropeptide in the neocortex involved in numerous processes. All cortical layers receive cholinergic inputs from the basal forebrain, which are crucial for cognition. Cholinergic receptors are differentially distributed across the cortex and the activation of nicotinic acetylcholine receptors (nAChRs) located on principal neurons and GABAergic interneurons (GINs) modulates synaptic plasticity and behavior. Functional consequences of the cholinergic activation of NPY-expressing cortical interneurons (NPY+GINs) haves not been specifically clarified yet. By using the CRISPR/Cas9 approach, we deleted the most abundant beta2* subunit of nAChRs expressed by NPY+GINs in the prefrontal cortex (PFC) and investigated the effect on behavior. By in situ hybridization, we characterized the expression of beta2* nAChRs on NPY+GINs. To induce a cell specific deletion of beta2* nAChRs, we crossed a mouse line expressing Cas9-GFP in a Cre-dependent manner with a NPY-Cre line. The resulting mice were injected with AAV vector carrying sgRNA targeting CHRNB2, gene coding for beta2 nicotinic subunit, in the prefrontal cortex. Mutant mice and littermates controls, injected with scrambled gRNA, were tested in a battery of behavioral tasks including social preference task and novel object recognition, followed by immunohistochemical evaluation of neuronal activity indicators.The successful induction of indels in CHRNB2 gene in AAV-transduced cells was confirmed by FACS sorting followed by DNA sequencing. Mice with presumed deletion of nAChRs showed increased social interaction compared to controls. The achieved efficiency of CRISPR/Cas9-induced indels in CHRNB2 gene in NPY+GINs of the PFC was sufficient to induce behavioral changes in mice. Specifically, the increased sociability agrees with Avale et al., 2011. This work was supported by the Grant Agency of the Czech Republic Grant #19-07983Y.

The role of lateral hypothalamic GABAergic neurons in encoding different stimuli outcome associations.

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Learning about environmental cues that predict positive or negative outcomes is essential for the production of adaptive behaviours. However, the positive or negative properties of these cues can in some cases be wrongly attributed, leading to maladaptive responses characteristic of disorders such as addiction, where the positive value of a drug memory persists over time even when the individual stops drug consumption, leading to relapse. Lateral hypothalamus (LH) is a brain region critical for memory and motivation. Recent studies suggest the GABAergic subpopulation of neurons in LH are key players in memory processes that involve different stimuli-outcome associations, although the specific mechanisms through which LH-GABA encode and express memories are still understudied. In this study, we aim to describe how alcohol reward memories are encoded and expressed in LH GABAergic neurons. We recorded calcium transients during a pavlovian conditioning tasks using fibre photometry. To target LH-GABA, we used a dual virus approach where we injected a mixture of two AAV encoding GAD-cre and cre-dependent gCaMP7f into the LH of Long-evans. We first trained the rats on an alcohol conditioning task in which rats learned to associate one conditioned stimulus (CS+, e.g. a light) with alcohol availability in a magazine (20% EtOH in water), while a different conditioned stimulus (CS-, e.g. a tone) was presented without consequence. Rats learned to respond to alcohol availability by spending more time in the magazine during CS+ compared to CS- trials. We then used an extinction protocol in which CS+ and CS- were presented without consequence. Rats significantly decreased their time spent in the magazine in response to the CS+ to that of levels of the CS-, thereby showing extinction. To test whether responding to the CS+ would reinstate, we primed rats on a subsequent single fluid exposure session, in which the alcohol was delivered in the same schedule as in conditioning sessions, but no cues were presented. Reinstatement of the behaviour was then tested by presenting both cues without alcohol the day after. We monitored LH-GABA activity throughout each phase. Results show LH-GABA neurons initially respond to non-conditioned, neutral cues. These responses are modulated throughout conditioning. Increased activity is observed to the alcohol predictive CS across conditioning sessions, alongside increased alcohol seeking. In contrast activity to the CS- remains unchanged or decreases. During extinction, LH-GABA activity in response to the CS+ decreases, while CS- data remains unchanged. Finally, while we did observe reinstatement of alcohol seeking, there was no change in LH-GABA activity. These data show that LH-GABA neurons are involved in the acquisition of new alcohol memories, but not after extinction. Future research shoud focus on monitoring the responses of these neurons to negatively valanced cues (e.g. fear conditioning), and also the interaction of these with alcohol reward cues. Understanding this interaction is pivotal to our understanding of addiction.

Habituation to repeated playback of 50-kHz ultrasonic vocalization in rats: Role of strains, pharmacological manipulations, and response calls.

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Rats communicate via ultrasonic vocalizations (USV) of different frequencies. Calls with a frequency around 50 kHz are thought to represent a positive affective state. During playback of such USV, rats emit a strong social approach towards the sound source. However, this approach behavior decreases during a second presentation days later, a phenomenon that is not well understood yet. The present behavioral experiments here were conducted in juvenile, male rats on a radial maze where 50-kHz USV playback was presented from one side. One week later, the animals underwent the same procedure in a retest. Approach behavior to 50-kHz USVs and possible response calls of the recipient were analyzed. We found that Sprague-Dawley, in contrast to Wistar rats did not habituate to 50-kHz USV since they kept approaching the sound source during the retest. Changing the internal state of the rat in either the first test and/or the retest pharmacologically with the dopaminergic antagonist haloperidol also prevented habituation. Rats also did not habituate when treated with the dopaminergic agonist d-amphetamine prior to the retest. Besides behavioral activation and approach, rats emitted USV in response to 50-kHz USV playback, but these calls were in a frequency range well below 50 kHz. Since these USV calls have not been investigated in detail yet, we characterized their occurrence and parameters. Among others, we found that they occurred frequently during the first test but were almost absent in the retest and we could see that they decreased if the animal habituated. Together, we could show that the habituation phenomenon is strain-dependent and can be prevented pharmacologically with dopaminergic drugs. As an additional indicator of habituation, we suggest measuring the emission of distinct USVs, since they decrease in an experience-dependent way. DFG funded.

An investigation of the effects of varying maternal resources on offspring physical development and movement in male and female Long-Evans rats.

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Maternal behavior is essential for the development of mammalian species; therefore, it is important to understand the impact of various stressors on maternal responsivity and offspring development. In the current study, the impact of varying levels of maternal resources on offspring development and movement (e.g., agility, exploration, and social play) was investigated. Long-Evans rats were assigned to treatment groups of standard or restricted resources (i.e., 25% of bedding and nesting materials) at parturition, so that maternal responsiveness and pup development could be assessed (n = 6/group). Pup developmental assessments were recorded throughout lactation indicating significantly shorter tail length and hind limb length in restricted pups until PND 14. Starting on PND 23, pup play and social behaviors were assessed for three days (5 mins/day). A significant interaction between sex and resource levels indicated that social grooming was lower in the restricted group males with no differences observed in the females; further, a significant interaction indicated that females had higher levels of self-grooming in the standard resource group with the males exhibiting lower scores in the low resource group. On the third day of testing, increased play bouts were observed in the low resource groups. To assess agility and balance, walking beams with varying characteristics were used. During the second trial, the standard animals fell off the rod more that the low resource animals. On a challenging translucent rod, significant interactions indicated that low resource females, with no differences observed in the standard animals. Histological investigations (targeting microglial responsiveness in the CA1 and amygdala, and hippocampal NPY) and endocrine assays (corticosterone and DHEA) are ongoing. In sum, the current findings indicate pervasive developmental effects of restricted postpartum maternal resources; further research is necessary to understand the underlying mechanisms associated with this rodent model of mild early-life stress.

Effects of neuropeptide S receptor deficiency and nasal administration of neuropeptide S on discrimination and reversal learning.

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Cognitive flexibility refers to the ability to modify learned behavior in response to changes in the environment. In laboratory rodents, cognitive flexibility can be assessed in reversal learning, i.e. the change of contingencies, for example in T-maze discrimination learning. The present study investigated the role of the neuropeptide S (NPS) system in cognitive flexibility. In the first experiment, mice deficient of NPS receptors (NPSR) were tested in T-maze discrimination and reversal learning. In the second experiment, C57BL/6J mice were tested in the T-maze after nasal administration of NPS. Last, the effects of nasal NPS on locomotor activity were evaluated. T-maze discrimination and reversal learning were not affected by NPSR-deficiency. Importantly, nasal NPS administration facilitated reversal learning and supported an allocentric learning strategy without affecting acquisition of the task and locomotor activity. Taken together, the present data indicate that the NPS system is a modulator of cognitive flexibility and suggest that nasal NPS administration could be a treatment option in diseases with impaired cognitive flexibility.

The effect of acute food deprivation on heroin seeking after short and long punishment-imposed abstinence, in male rats.

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Heroin addicts often self-impose abstinence due to the negative consequences of drug use. Punishment-imposed abstinence is an animal model that introduces negative consequences (e.g., mild footshock) with drug seeking or taking. Acute food deprivation (FD) is a stressor that has been shown to significantly reinstate extinguished heroin seeking. However, acute FD effect was never studied after punishment-imposed abstinence. Studies using punishment-imposed abstinence usually use 5 to 10 days of punishment, therefore, little is known about longer abstinence periods using punishment. It has been demonstrated that longer abstinence periods lead to higher drug craving (an effect termed incubation of drug craving). Here we investigated the effect of acute FD on heroin seeking after 8 and 21 days of punishment. Self-administration training was done under the Seek-Take chain protocol. Rats pressed the Seek lever under a variable interval of 60 s to get access to the Take lever that was paired with discrete cues and a heroin infusion (0.1 mg/kg/infusion). After 2 weeks of self-administration training, footshock was delivered on 30% of completed Seek links, instead of Take lever access. One group (N=24) underwent 8 days of punishment-imposed abstinence with the footshock intensity increasing 0.1 mA per day, from 0.2 mA to 1 mA. A second group (N=9) underwent punishment-imposed abstinence for 21 days, with footshock intensity increasing 0.1 mA/day until it reached 0.6 mA, and it remained at 0.6 mA for the rest of the abstinence days. Next, rats were tested for heroin seeking following 24 hr FD and sated conditions, in a counterbalanced order. FD significantly increased heroin seeking after 8 days (p=.0008, d=0.92) and 21 days of punishment (p=.014, d=0.45). In conclusion, acute FD augmented heroin seeking after short and long punishment-imposed abstinence.

Activation of a cortico-thalamic neural circuit attenuates renewal in male and female rats.

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Contexts associated with prior reinforcement can renew extinguished conditioned responding. Both the infralimbic medial prefrontal cortex (IL) and paraventricular nucleus of the thalamus (PVT) are implicated in this renewal effect. Here we examined if connectivity between these regions, namely an IL-to-PVT neural circuit, was involved in renewal. We used optogenetics to stimulate the IL-to-PVT neural circuit during ABA renewal of Pavlovian conditioned responding to a sucrose-predictive cue. Briefly, we trained male and female Long-Evans rats (Charles River) to associated a conditioned stimulus (CS; 10 s white noise) with the delivery of a 10% liquid sucrose (w/v) unconditioned stimulus (US; 0.2 mL/CS) into a fluid port for oral consumption in a distinct context (Context A; 14 trials/ session; 12 sessions). We then extinguished CS-elicited port entries by presenting the CS without the US in a different context (Context B; 14 trials per session; minimum of 2 sessions). At test, we used optogenetics to unilaterally stimulate IL neurons projecting to the PVT during CS presentations in the original training context (ABA renewal test) and in the extinction context (ABB control test) (473 nm; 5 ms pulses at 20 Hz for 10.2 s). Optically stimulating the IL-to-PVT neural circuit in rats expressing Channelrhodopsin-2 with enhanced yellow fluorescent protein (ChR2-eYFP) significantly attenuated ABA renewal of CS-elicited port entries compared to controls expressing eYFP alone, but had no effect on responding during the inter trial interval. This effect was equivalent in both males and females. Furthermore, rats expressing ChR2-eYFP (1) nose-poked significantly more for optical self-stimulation of IL-to-PVT compared to rats expressing eYFP alone, and (2) expressed greater Fos density (a marker of neural activity) in the IL and the PVT compared to rats expressing eYFP-alone. These results demonstrate a compelling, sex-independent role of the IL-to-PVT neural circuit in mediating renewal of appetitive Pavlovian conditioned responding following extinction. Funded by: Natural Sciences Engineering and Research Council of Canada (NSERC)

Identification of pain hypersensitivity across genetically diverse mouse lines.

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The same pain can cause wildly different reactions depending on who experiences it, and the key to understanding why could lie in the genetics that underlie the pain pathway. Genetic factors, such as genes coding for nociceptive sodium channels, which lead to hyper and hyposensitivity to pain through sensitivity to stimuli have previously been isolated, but the genetics which regulate how an animal responds to that stimuli are not well understood. Identifying these genes is essential to understanding the neural pathways that the brain uses to execute pain behaviors in response to noxious stimuli. Here we use the model of thermal escape jump behavior to examine how genetic variation leads to differences in pain behavior independent of pain sensitivity. We tested 18 genetically diverse inbred mouse lines to determine the levels of escape jump behavior they showed when place on a hot plate at either extreme hot or cold temperatures, and then tested them for thermal sensitivity to heat and cold for comparison. Our results found that C57/BL6 mice, which are commonly used in pain research, are an outlier in terms of thermal escape jump behavior when compared to non C57 strains. Comparisons with several strains showed decreases in this behavior which could not be explained by differences in thermal sensitivity. This testing found heightened jumping behavior without any significant difference in thermal sensitivity, with C57BL/10SnJ mice, a subline of C57 mice, showing more than 10 times the jumping behavior exhibited by C57/BL6 mice. Further when tested for general escape behavior using the tail suspension assay C57/10SnJ mice showed twice the escape behavior as C57BL/6J mice. Critically, these findings indicate that these differences in jumping behavior are not caused by changes in nociception but instead to the behavioral response itself. These results establish C57BL/10SnJ mice as targets for genetic analysis through which the specific neural circuitry underlying behavioral reactions to pain could be established. Keywords: Escape behavior, Pain, Thermal pain, genetics, ongoing pain behavior, acute pain behavior, Inbred mouse line. Funding Acknowledgments: IA-S and JB are supported by startup funds from the University of Pennsylvania and by a grant from the National Institutes of Health NIH/NIDCR, R00-DE026807

Work it out?: Does exercise rescue oxidative damage caused by maternal deprivation in female c57/Bl6J mice?

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Traumatic early life stress, including maternal deprivation (MD), can permanently alter neurodevelopment and produce negative effects such as inducing oxidative stress in the brain. This increases the susceptibility to psychopathology, impaired cognitive function, and stress related disorders over one's lifetime. These vulnerabilities are due to chronic dysregulation of the hypothalamic pituitary adrenal (HPA) axis causing inflammation and neurotoxicity. Males have consistently shown to have higher vulnerability to early life stress due to higher microglia in specific brain areas compared to females. However, sex differences and possible female resiliency is poorly researched, and there is little research regarding rescue interventions for MD. Exercise is potently neuroprotective in several stress models, yet no research has been conducted on the potential rescue effects of exercise on the well-known toxic effects of MD. Thus, we hypothesized that exercise will similarly rescue the toxic effects of MD in female mice. To assess these protective effects, c57/Bl6J mice were bred and female MD pups were weaned one week early on postnatal day (PND) 14, while standard weaning was on PND 21. At weaning, pups were paired and housed in either sedentary (SH; standard caging) or exercise (EX; standard caging with two running wheels). The expression of the resiliency factors brain derived neurotrophic factor (BDNF) and neuropeptide Y (NPY) were assessed in the CA1-3 regions of the stress vulnerable dorsal vs resilient ventral hippocampus. An ANOVA on BDNF expression in the dorsal hippocampus CA1-3 regions showed no significant mean effects of MD ($p= 0.982$), housing ($p= 0.808$), or an interaction ($p= 0.274$). Further work will investigate differences in expression of BDNF compared to the ventral hippocampus, as well as expression of Neuropeptide Y. Proinflammatory microglia will also be assessed via Iba-1 and Cox-2 staining in the hippocampus. Funding was provided by Schapiro Undergraduate Research Foundation, R-MC.

Colon misconduct: Early life stress disrupts the microbiome-gut-brain axis.

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Dysregulation of the gut-brain-axis is implicated in anxiety and gastrointestinal (GI) disorders. Importantly both anxiety and some GI disorders show differences in prevalence between males and females. Individuals who experience early life stress are more susceptible to the development of both anxiety and GI disorders in later life. However, the mechanisms underlying the development of these disorders remains to be established. We used our neonatal immune activation model to investigate the lasting effect of early life stress on behaviour, colon inflammation and the microbiome. Wistar rats were injected with LPS or saline on postnatal days 3 and 5. In adulthood, behaviour tests were performed to assess anxiety-like behaviour and colons were collected to assess inflammation, and microbiome composition. There were distinctly different phenotypes for LPS-exposed males and females. LPS males displayed typical anxiety behaviours with decreased social interaction, and increased defecation relative to controls. LPS-exposed females displayed more adaptive behavioural phenotype with increased social interaction and exploration compared to controls. For microbiome profiling data, Bacteroidota was significantly increased for LPS females and Proteobacteria was decreased for LPS rats of both sexes. Further, LPS-exposed females matched the microbiome composition of control males. Histological analyses showed that LPS-exposed males displayed increased colon inflammation with increased lymphocyte infiltration, an effect that was not seen in exposed females. We have shown that early life stress leads to both behaviour and gastrointestinal changes, and that these alterations are sex dependent. These findings highlight the importance of sex in determining the impact of early life stress on the gut-brain-axis.

Association of infant outcomes with brain-derived neurotrophic factor (BDNF) levels at birth.

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Brain-derived neurotrophic factor (BDNF) is crucial to healthy brain function. Significant amounts of this growth factor are also found in the blood, stored in circulating blood platelets, however the function of this source of BDNF is not yet known. Despite this, altered levels of BDNF in serum (a blood derivative) are consistently linked with neurological disorders, including depression. BDNF is increasingly implicated in the pathogenesis of attention-deficit hyperactivity disorder (ADHD), a developmental disorder much more frequent in males. At birth, male infants naturally have significantly lower levels of serum BDNF than female infants (~15-20% lower), which may render them more vulnerable to neurodevelopmental disorders, particularly in the context of early life adversity. We previously characterized serum BDNF levels in mothers (n=251) and their newborn infants (n=212) as part of the Grown in Wales Study. Here, we assessed the development and temperament of the Grown in Wales infants between 12-14 months, using the Laboratory Temperament Assessment Battery (Lab-TAB) to assess parameters relevant to symptoms of ADHD. We identified a significant positive relationship between infant serum BDNF levels at birth and facial interest scores during the sustained attention task, a relationship that was significant in male infants only. This sex-specific association between serum BDNF levels at birth and improved parameters of attention at 12-14 months supports the hypothesis that the reduced levels of serum BDNF seen in infant males at birth may contribute to their increased risk for ADHD and other neurodevelopmental disorders. Funding is gratefully acknowledged from The Waterloo Foundation (HD: Child Development Fund Research Grant No. 1403-4535; KAS: 918-3022), from the MRC (SMG: GW4 BioMed PhD Studentship MR/N013794/1), from the BBSRC (LAS: SWBio PhD Studentship BB/M009122/1) and the Sêr Cymru programme (XN, YAB). The Grown in Wales Study was funded by MRC grant MR/M013960/1.

Exercise protects against the toxic effects of chronic stress in female c57/Bl6J mice.

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Chronic psychological stress induces oxidative stress, stimulating the brain to produce damaging reactive microglia, which have been linked to neurodegenerative diseases in both human and animal studies of chronic stress. Research indicates that exercise mitigates the toxicity of reactive microglia, possibly by increasing the brain's resiliency to oxidative stress. Notably, there are sex differences in the reactivity with microglia, yet there is little research utilizing female models. This research investigated if chronic stress creates a toxic microenvironment in the brain, as well as the effect of exercise within the female brain. To accomplish this, female c57/B16J mice were pair-housed with either sedentary standard housing (SH) or standard cages equipped with two exercise wheels (EX) and assessed after a two-week acclimation period either under exposure to chronic restraint stress (CRS), opposed to no stress (NS), for a randomized two hours daily for an additional two weeks. Following the final stress session, brains were collected and analyzed for markers and resiliency. By quantifying brain-derived neurotrophic factor (BDNF) and neuropeptide Y (NPY) in the CA1-3 regions of both the stress-vulnerable dorsal hippocampus and the stress-resistant ventral hippocampus. ANOVA showed no main effects of stress treatment (p= 0.649), housing (p=0.997), or interaction (p=0.103) on NPY and BDNF treatment (p=0.510), housing (p=0.488), and interaction (p=0.084) expression between CA regions. We are continuing analysis of resiliency factors between ventral and dorsal regions, additionally, we intend to expand our analysis to include the central amygdala. This research was funded by a Randolph-Macon College Chenery Research Grant.

Functional connectivity maps for recent and remote contextual fear memory retrieval

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The amygdala, anterior insular cortex (aIC) and anterior cingulate cortex (ACC), are part of the Salience Network (SN), and are highly responsive to arousing events in humans and rodents. It is thought that strong, aversive events prompt a shift of memory encoding dependence from a neocortical-hipocampal circuit to SN regions during acquisition. We explored whether there are changes in functional connectivity and FOS+ activity of brain regions associated with the SN after recent or remote retrieval of contextual fear conditioning (CFC) in rats. We used freezing as measure of conditioned fear. We saw that mild CFC (0.3mA) elicited discriminative freezing at 28 days (remote), whereas stronger CFC (1.0mA) elicited generalized fear at this timepoint. FOS expression across 8 brain regions allowed us to survey functional connectivity between them following memory recall at a recent (2 days) or remote timepoints. Our results showed that, hippocampal functional connectivity remained stable over time, but there was a shift of connectivity of the hippocampal subfields (CA1, CA3 and Dentate Gyrus) from the basolateral nucleus of the amygdala (BLA) and Retrosplenial Cortex granular (RSCg) to the ACC and the aIC. Moreover, there was an increase in connectivity between the ACC to the BLA and the aIC, and between the pre-limbic cortex (PL) to the aIC at the remote timepoint. Likewise, there was an increase of FOS+ cells in the aIC and PL only for the stronger CFC group tested at the 28th day. Remote freezing times were positively associated with generalized fear whereas the ratio DG/CA3 showed a negative correlation with freezing. These results suggest that higher arousal during memory acquisition elicited differential processes of systems consolidation, reorganizing brain-wide retrieval dependence from a BLA-hippocampus-RSCg network to an ACC-PL-aIC-amygdala one, favoring contextual fear generalization. This evidence agrees with the hypothesis that stress changes memory system dependence from more cognitive to habit and emotional memories. Funding from the Sao Paulo Research Foundation, grant #2017/24012-9 and #2017/03820-0.

Role of orexin in cognitive flexibility, sensorimotor gating and impulsivity.

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Deficits in cognitive flexibility, impaired sensorimotor gating and increased impulsivity are behavioral endophenotypes of several neuropsychiatric disorders including schizophrenia and attention-deficit/hyperactivity disorder (ADHD). Several classical transmitter systems including dopamine and noradrenaline are shown to be involved in these behavioural endophenotypes, however, there is not much known about the role of neuropeptides. The orexin neuropeptides, which are brain-widely released by neurons of the lateral hypothalamus, are major players in maintaining sleep/wake cycle, feeding behavior, arousal, and motivational behavior. Disruption of orexin signaling in basal forebrain impairs attention and cognition. Recently, we showed a sex-dependent modulation of cognitive flexibility in homozygous orexin-deficient mice. To further investigate the role of orexin in cognitive flexibility, we acutely blocked OX1R brain wide in male and female C57BL/6J mice and tested them in the attentional set shifting task (ASST). We found that females were impaired in reversal phases but not males. Furthermore, we tested attention and impulsivity in male and female orexin-deficient mice in the 5-choice serial reaction time task (5-CSRTT) using an automated touch screen set-up during both light and dark phase. We observed increased impulsivity in females but decreased impulsivity in male homozygous orexin-deficient mice. This indicates a sex-dependent role of orexin in both cognitive flexibility and impulsivity and urges the need for more sex-specific research and treatment strategies for the symptoms of neuropsychiatric disorders.This work is supported by the DFG (SFB779/B13 and FE 483/10-1).

Comprehensive characterization of motor and coordination functions in three adolescent wild-type mouse strains

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Neuropsychiatric disorders are often associated with motor and coordination abnormalities that have important implications on the etiology, pathophysiology, and management of these disorders. Although the onset of many neuropsychiatric disorders including autism spectrum disorder, schizophrenia, and attention-deficit hyperactivity disorder emerges mainly during infancy and adolescence, most of the behavioral studies in mice modeling neuropsychiatric phenotypes were performed in adult animals, possibly missing valuable phenotypic information related to the effect of synaptic maturation during development. Here, we examined which behavioral tests assessing both motor and coordination functions can be performed in mice at two different adolescent stages. As strain and sex affect mouse behavior, our experiments covered both male and female mice of three inbred wild-type strains, C57BL/6N, DBA/2, and FVB/N.Adolescent mice of both postnatal days (P)22-30 and P32-40 developmental stages were capable of mastering common motor and coordination tests. However, results differed significantly between strains and sexes. Moreover, the 10-day interval between the two tested cohorts uncovered a strong difference in the behavioral results, confirming the significant impact of maturation on behavioral patterns. Notably, the results of distinct behavioral experiments were directly correlated with the weight of mice, which may explain the lack of reproducibility of some behavioral results in genetically-modified mice. Our study paves the way for better reproducibility of behavioral tests by addressing the effect of the developmental stage, strain, sex, and weight of mice on achieving the face validity of neuropsychiatric disorder-associated motor dysfunctions.

Role of orexin deficiency in polydipsia and binge-like eating behavior.

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Since its discovery, the orexin neuropeptide system is known for its crucial role in regulating ingestive behaviors. Here, we focus on the involvement of orexin in polydipsia (excessive water drinking) and binge-like eating behavior. For that, the effects of heterozygous and homozygous orexin deficiency in female and male mice were tested in two behavioral experiments: (1) Drinking behavior after intracerebroventricular (ICV) injections of the dipsogenic peptide angiotensin II (ANG II), and (2) binge-like eating in a paradigm in which mice had intermittent access (24-h, weekly) or continuous access to a high-energy diet (HED) for three weeks, followed by testing for anxiety-like behavior. Experiment (1): ICV ANG II injections (100 ng) stimulated water intake in male mice, but not in female mice. These effects were independent of the orexin genotype. However, a higher dose of ANG II (500 ng) also induced drinking in female wild-type mice, but not in female orexin-deficient mice. Therefore, the dipsogenic effects of ANG II are sex-dependently influenced by orexin deficiency. Experiment (2): In the intermittent group, mice of all genotypes and sexes consumed significantly more food during the weekly 24-h HED access compared to the continuous control group. Moreover, mice of the intermittent group expressed increased levels of anxiety. Preliminary data suggest that orexin deficiency affected binge-like eating in a sex-dependent manner, i.e., it reduced binge-like eating in females, but not in males. Furthermore, the increased anxiety-like behavior observed in the intermittent group seemed to be mainly driven by males of each genotype, while female orexin-deficient mice partly did not show higher levels of anxiety. In conclusion, our results indicate and further substantiate an important and sex-dependent role of orexin in ingestive behaviors, which may be of translational relevance for human conditions like polydipsia and binge eating disorder. This work is supported by the DFG (FE 483/10-1).

Valproic Acid Alters Sociosexual Signalling in Male Naked Mole-rats During their Pubertal Transition.

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Adult neuroplasticity is an adaptive process by which animals can alter behavior in response to shifting environmental stimuli. Social neuroepigenetics explores the interplay between epigenomics and the social environment. The naked mole-rat (NMR) presents a fascinating opportunity to examine this relationship. NMRs exhibit the most extreme form of socially-mediated reproductive suppression (eusociality). They reside in large colonies where the only reproductively-active animals are a single breeding female and 1-3 male consorts; all other animals are pre-pubertal and socially subordinate. Most NMRs never go through puberty unless removed from the suppressive social cues of their colony. Valproic acid (VPA) is a histone deacetylase inhibitor that alters social behavior in diverse species (e.g. alters caste-related behavior in carpenter ants, enhances social defeat in hamsters). Drugs of a similar class are also implicated in control of pubertal onset in rodents. To test the hypothesis that DNA acetylation is involved in NMR reproductive/neural plasticity, we removed animals from their home colony for 1 week to trigger puberty onset and, simultaneously treated them with either peripheral VPA injections (twice daily) or vehicle control. Animals were scored for sociosexual behaviors with an unfamiliar opposite-sex conspecific prior to/following colony separation and VPA manipulation. VPA-treated males received increased body/genital investigation from stimulus females compared to saline-treated males; VPA treatment did not affect investigatory behaviors received by females. Preliminary protein quantification of histone 3 acetylation marks revealed increased acetylation of H3K18 in VPA-treated males, but not females. Finally, VPA-treated males had increased genital size compared to controls. Collectively, these data demonstrate altered sociosexual signalling in male, but not female, NMRs treated with VPA during their pubertal transition, suggesting a key role for acetylation in at least some components of socially-induced plasticity observed in this species. MFM was supported by a NSERC PGS-D/CGS-D, ACM/Intel Computational Science Fellowship; MMH was supported by NSERC grants (RGPIN 2018-04780/RGPAS 2018-522465).

Impaired cognitive flexibility, fear and safety learning in Shank2-deficient mice.

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Shank2 is an excitatory postsynaptic scaffold that is important for synaptic protein assembly and NMDA receptor related signaling. In humans, Shank2 mutations are associated with several neuropsychiatric disorders. Shank2-deficiency in mice results in behavioral endophenotypes of neuropsychiatric diseases such as social deficits, repetitive behavior, and anxiety-like behavior. Of note, these mice also show a NMDA receptor hypofunction, and pharmacologically restoring NMDA receptor function improves some of the behavioral deficits. The aim of the present study was to assess the role of Shank2 in cognitive flexibility and associative learning processes. Hence, Shank2-deficient mice were tested in the attentional set shifting task (ASST) and in a fear/safety learning task. Shank2-deficient female (but not male) mice showed an overall ASST performance deficit that was most pronounced in the reversal phases. Treatment with the partial NMDA receptor agonist D-cycloserine rescued this ASST deficit. Furthermore, we found impaired contextual fear and safety learning in female and male Shank2-deficient mice. In addition to the behavioral experiments, we started to analyze the expression pattern of components of NMDA receptor related signaling pathway â€“such as different subunits of the NMDA receptor, serine racemase, and phosphoglycerate dehydrogenase (PHGDH)â€“ in different subregions of the frontal cortex which are critical for the ASST or fear/safety learning. Taken together, our data demonstrate an important role of Shank2 in cognitive flexibility measured by the ASST and in contextual fear and safety learning. Furthermore, first data from molecular analyses as well as the rescuing effects of D-cycloserine indicate that the observed effects of Shank2-deficiency are associated by changes in NMDA receptor signaling.

Neural and behavioural analyses of retrospective and prospective fear triggers

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Memories about aversive events (e.g. trauma) that elicit fear responses (i.e. primary triggers of fear) can propagate across the memory network, linking fear to other stimuli (i.e. secondary triggers of fear). This process can occur retrospectively and prospectively and is captured by sensory preconditioning and second-order conditioning, respectively. In sensory preconditioning two sensory stimuli are paired before one of those stimuli is paired with foot-shock. In second-order conditioning, sensory pairings occur after fear conditioning of one of the stimuli. In a series of studies we show that pharmacological inactivation of the orbitofrontal cortex (OFC) prior to test for fear to the higher-order cues disrupts fear to the sensory preconditioned cue but enhances fear to the second-order cue. An investigation into the role of OFC input to the BLA and BLA input to the OFC during higher-order learning revealed that silencing OFC input to the BLA disrupted sensory preconditioning but not second-order conditioning. Silencing BLA input to the OFC, however, disrupted both effects. These results uncover a novel role for the OFC in higher-order fear and elucidate the interaction between the OFC and BLA in this learning. This work was supported by; a CIHR Project Grant (to MDI); the Canada Research Chairs program (to MDI Grant 950-230456); a FRQS post-doctoral fellowship (to BPPL).

Transgenic Manipulation of miR-137 Activity in the Dorsal Striatum Promotes Compulsive Cocaine Seeking

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Small non-coding microRNA (miRNA) play a key role in post-transcriptional modification of gene expression. In the nervous system, microRNAs control basic functions including neuronal development, synapse formation and long-term plasticity. Previous work in a rodent model has shown that miR-137 is differentially expressed in subregions of the dorsal striatum at different phases of the addiction cycle. As miR-137 is understood to be a known negative regulator of neuronal functions, it was hypothesised in the present study therefore, that transgenic manipulation of miR-137 activity may influence compulsive drug-seeking through the regulation of genes linked with synaptic plasticity. Male Sprague-Dawley rats (n = 35) were trained to lever press for intravenous cocaine hydrochloride (0.25 mg/ml) infusions in daily 2h self-administration sessions. After animals had acquired a stable pattern of cocaine self-administration, rats were randomly allocated for stereotaxic injections of lentivirus expressing the appropriate transgene in the dorsal striatum. Post-surgery, rats were returned to daily cocaine self-administration sessions. To test for compulsive drug-seeking, lever presses were associated with random delivery of 0.5 mA foot shocks. Following reinstatement of normal responding, rats completed two progressive ratio (PR) sessions to assess motivation to work for cocaine. Rats were euthanised and perfused for RNA-sequencing analysis. Viral-mediated over-expression (miR-137OE) was confirmed in a separate cohort (n=3 per time point) and revealed a significant increase in fold change for pre-miR137 at 3 and 6 weeks post-injection. Rats receiving miR-137OE (n = 16) maintained cocaine self-administration despite punishment for five days and received, on average, more shocks per session than controls, maintaining their pre-virus cocaine consumption. Control animals (n = 19) on average declined their cocaine consumption steadily each day. The present results support a link between dysregulated expression of miR-137 and compulsive drug-seeking. While further work is required to understand how miR-137 alters striatal function and contributes to compulsive drug-seeking, it is suspected that target genes associated with synaptic plasticity are involved. These findings may give insight into the mechanisms linking schizophrenia with addiction and hopefully provide a better understanding of the neural substrates contributing to these disorders.

Immediate Post Training Inactivation of the Anterior Cingulate Cortex Blocks Recent, but not Remote Fear Generalization

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A hallmark of many anxiety disorders as well as trauma- and stressor-related disorders is the overgeneralization of fear. This is characterized by the expression of fear in new or non-threatening environments and can disrupt normative functioning. We have previously found that the Ventral Hippocampus (vHPC) and Anterior Cingulate Cortex (ACC) regulate generalized fear through their efferent connections to the Basolateral Amygdala (BLA). Specifically, inactivating ACC or vHPC projections to the BLA significantly reduced generalized fear to a novel, nonthreatening context but had no effect on fear to the training context. Further, we showed that the ACC-BLA circuit supports generalization in a time-independent manner, whereas the vHPC-BLA circuit plays a strictly time-dependent role in supporting remote generalized contextual fear. The current experiments aim to investigate the ACC's role in supporting immediate generalization. Mice were trained using a contextual fear conditioning procedure that produces generalization within 24 hours. Immediately after training, mice received local infusions of lidocaine (4%) or vehicle (PBS), into the ACC. The mice were then tested either 1 day or 28 days later in the same context where the training occurred or in a novel, but non-threatening context. We found that inactivation of the ACC immediately after training blocked generalized fear during the recent (1 day) test, but left fear to the training context intact. However, post-training inactivation of the ACC had no effect on generalized fear at the remote (28 day) test. These findings suggest that the ACC is involved in consolidation processes supporting immediate generalization but may be dissociated from those processes that support remote generalized fear.

The affective bias test: A refined and translational method for assessment of novel antidepressants.

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For more than 40 years the favoured pre-clinical methods for research into antidepressants (ADs) have been forced swim test or tail suspension test. Whilst these have been useful in the development of many of the ADs treatments used today, they have limited translational validity. To try to address this we developed a new behavioural assay derived from human neuropsychology. Our aim was to recapitulate in animals the clinical observations that emotional state can bias cognition, termed affective biases. Specifically, depressed patients have been shown to exhibit negative affective biases whilst acute treatment with ADs has been shown to induce positive biases in healthy volunteers and patients. The affective bias test (ABT) is a bowl digging task designed to measure affective state-induced biases related to reward learning and memory. The task requires animals to learn two independent cue-reward associations (digging in a specific substrate to obtain food reward). Value of each experience is the same but affective state can be manipulated prior to one of the experiences. Using a choice test the animal is then 'asked'; which do you prefer. Using a wide range of drug treatments and psychosocial manipulations, including early life adversity, we have demonstrated face, construct, predictive, homological and translational validity, with medium to large effect sizes. The ABT studies also suggest that conventional ADs vs rapid-acting ADs differentially modulate affective biases with the assay able to dissociate between these different AD classes.

Evidence for the existence of an entourage effect in Psilocybe mushrooms

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Entourage effect refers to the idea that multiple chemicals may be active simultaneously in an abused substance, eliciting different effects. To address the possibility of an entourage effect in psilocybe "magic"; mushrooms, we examined the behavioral effects of psilocybin and norbaeocystin, individually and in combination. Psilocybin and norbaeocystin were synthesized using genetically modified E. coli and verified for purity using mass-spectroscopy. Varying doses of filtered E. coli broth containing each compound were then gavaged into male rats, and head twitch responses and locomotion were assessed. Dose-dependent head twitch responses were exhibited following the administration of psilocybin alone, but not norbaeocystin. Additionally, the number of head twitches produced by psilocybin was increased when it was combined with norbaeocystin, beyond simple additive levels, suggesting pharmacological interaction may be occurring. Combined, our data demonstrate the efficacy of E. coli-derived psilocybin and suggest that other tryptamines may supplement/augment psilocybin's efficacy. Additionally, our results demonstrate the relative safety of gavaging filtered E. coli broth containing these compounds. Preliminary clinical research has shown that psilocybin may have potential therapeutic utility for the treatment of depression, anxiety, PTSD, and substance use disorders. Thus, future studies will assess the therapeutic potential of both compounds, as well as determine their mechanisms of interaction.

The long-lasting impact of early-life stress on reward-processing deficits: A systematic review and meta-analysis of evidence from animal models.

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The effects of early life stress (ELS) on reward-processing in adulthood have been extensively investigated in rodents. Evidence suggests that timing of ELS during critical neurodevelopmental windows (e.g. neonatal vs. adolescent periods) might differentially influence reward-processing profiles in later life. However, there are inconsistencies within this literature; while the majority of studies observe anhedonic-like reductions in adult rodent's reward sensitivity following ELS, others report heightened sensitivity to rewards. These conflicting findings might arise from sex differences, type of ELS, and other methodological factors. Hence, we conducted a systematic review and meta-analysis to assess the impact of ELS during the neonatal or adolescent period on reward-processing profiles in the sucrose preference (SP) test. We further examined whether SP outcomes are influenced by sex and ELS modality. We performed a literature search using PubMed database through December 2020. Included studies were published, employed rat or mouse models, with subjects exposed to ELS and tested for SP in adulthood. Ultimately, 48 experiments (n=1108) met the predetermined inclusion criteria. The overall effect of ELS on SP scores in adulthood was significant, with ELS rats showing decreased SP (reward hyposensitivity) relative to non-stressed controls. In contrast to our predictions, the timing of ELS (neonatal vs. adolescence) did not significantly alter SP outcomes. Subsequent subgroup analysis revealed that sex did not have a significant effect on SP outcomes, nevertheless, ELS significantly reduced male's SP scores, whereas ELS did not significantly alter SP scores in females. Interestingly, further subgroup analyses revealed a significant effect of ELS modality on SP. Ultimately, these findings suggest that type of ELS, rather than timing of ELS, might influence diverging reward-processing profiles in adulthood. This knowledge will help to guide future research studying the long-lasting impact of ELS on varying reward-related constructs. Research supported by the Natural Sciences and Engineering Research Council of Canada.

Exploring the emotional blunting domain of apathy in aged mice

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Apathy is a complex neuropsychiatric symptom that is a common feature of neurodegenerative disease but also otherwise-healthy aging. It is associated with reduced quality of life and significant caregiver stress. Emotional blunting is a core feature of apathy but is often overlooked in favour of effort-based paradigms when probing for apathy behaviour in rodent models. Given its multidimensional nature, testing of apathy requires a multifactorial approach that considers the mechanism of apathy at multiple levels, including the emotional response. The aim of the present study was to assess emotional reactivity in aged mice. 12 male C57bl/6J mice aged 15 mo at experiment onset and 24 mo at experiment end were kindly supplied by Eli Lilly & co. 12 strain and sex matched controls aged 3 mo at experiment onset and 12 mo by experiment end were supplied by Charles River. Mice underwent a battery of behavioural tests hypothesised to map onto different domains of apathy. Here, we only report the behavioural tests pertaining to emotional behaviour. Anxiety-related behaviour was assessed using the novelty supressed feeding test (NSFT) and reward sensitivity was assessed using the sucrose preference test (SPT). Using 3 age groups of approx. 3 mo, 12 mo and 24 mo, stress reactivity was further assessed by measuring the corticosterone (CORT) levels in the blood in response to restraint stress as well as cfos expression in the paraventricular nucleus of the hypothalamus (PVN) and amygdala. Following analysis, there was a number of statistically significant findings (p<0.05). Aged mice were quicker to eat in the NSFT but had a reduced sucrose preference in the SPT indicating reduced stress-induced anxiety and reduced reward sensitivity respectively. The older groups had a blunted CORT level post-restraint stress compared to the younger group, indicating reduced stress reactivity. The oldest group had reduced activation of the PVN compared to the younger group but greater activation of the amygdala. Overall, these studies provide evidence of changes in emotional reactivity, reward sensitivity and stress reactivity consistent with emotional blunting and possibly apathy in aged mice. This work was funded by the BBSRC SWBio DTP and Eli Lilly & Co.

Neural Characteristics of Captive Raccoons in a Reversal Learning Task.

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The raccoon (Procyon lotor) has demonstrated impressive adaptive skills in the wild and cognitive flexibility skills in laboratory studies. These animals are of recent interest to our lab as an alternate preclinical model to the common lab rat, given their gyrified brains and other shared neurological characteristics with primates. Wild-caught raccoons maintained in captivity were selected based on likelihood of voluntary participation, as demonstrated by lower anxiety-like phenotypes and a high degree of food-motivation. These animals were exposed to a reversal learning task, in which they had to demonstrate associative operant learning on one active panel before reversing their strategy to respond to the alternate panel for a food reward. "Solver" raccoons (N=5) demonstrated reversal learning, while "nonsolver raccoons" (N=3) failed to reach the reversal criterion. Fecal samples were collected for stress hormone analyses. Subsequently, brains were collected for histological processing. Targeted neural characteristics were evaluated using isotropic fractionation (IF) methods as well as IHC staining. In solver raccoons, the DHEA:CORT ratio was significantly higher than nonsolver raccoons (p=0.04), suggesting stress hormones may have contributed to performance results. IF analyses indicated higher average total cell counts in the hippocampus (HC) of solver animals (X=42,451,250) were higher than nonsolvers (X=33,231,250); however, no significant differences were found. Interestingly, the percent neuron estimates in the HC were similar between groups, indicating greater total cell counts could be due to glial cells. Preliminary IHC results for the NMDA receptor subtypes, GRIN2A and GRIN2B, suggest a greater 2B:2A ratio in the solver raccoons. The ratio of higher 2B to 2A expression has been implicated in adaptive flexible cognition, and is one potential mechanism underlying differential performance on this reversal task. Additional research with this model is necessary to learn more about neurobiological mechanisms associated with cognitive flexibility.

Application of the NIH Toolbox to assess neurocognitive function in individuals with cooccurring serious mental illness and alcohol use disorder.

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Forty-six percent of individuals with a serious mental illness (SMI; i.e., schizophrenia spectrum, bipolar, major depressive disorder) have a cooccurring alcohol use disorder (AUD). Neurocognitive impairments are associated with SMI and AUD individually, and are believed to contribute to the chronic nature of these conditions and poorer treatment outcomes. However, characterization of neurocognitive function in individuals with cooccurring SMI and AUD (SMI-AUD) is less established. The current study used the NIH Toolbox Cognitive Battery to assess the prevalence of mild cognitive impairment (MCI) in individuals with SMI-AUD in addition to investigating psychological and alcohol-related predictors of MCI. Methods: 107 outpatient treatment-seeking individuals with SMI-AUD were enrolled. The NIH Toolbox was administered at the baseline interview and measured language, working memory, episodic memory, processing speed, and executive functions. The presence of SMI and AUD severity was assessed using the SCID-5. Additionally, a urine sample was collected to evaluate recent drinking by measuring the alcohol-related biomarker ethyl glucuronide (uEtG). The presence of MCI was established using the Holdnack equation. Logistic regression models were applied to examine associations between psychological (e.g., SMI type, positive/negative symptoms) and alcohol-related (e.g., uEtG level, AUD severity) predictors of MCI. Results: Forty-two percent of our SMI-AUD sample had an MCI. Of the NIH Toolbox subtests, those with MCI exhibited neurocognitive impairments in working memory (score = 45.6, CI = 43.5, 47.7) and executive functions (score = 39.6, CI = 37.8, 41.5). There was an association between negative symptoms and MCI (OR = 1.10, CI = 1.01, 1.21). We also found that AUD severity moderated the effect of recent drinking level on the likelihood of MCI (OR = 1.05, CI = 1.02, 1.09). Conclusion: MCI is highly prevalent in individuals with SMI-AUD. Future studies will investigate if MCI is associated with treatment outcomes in individuals with cooccurring SMI-AUD. Funding: NIAAA R01AA020248

The effects of naltrexone on the abuse potential of intranasal methamphetamine.

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The current trial aimed to examine the effects of acute NTX pretreatment on the abuse potential of intranasal methamphetamine. Healthy male and female, non-medical psychostimulant users between the ages of 21 and 50 years, were recruited for this randomized, placebo-controlled, within-subjects trial. Participants completed test sessions in which oral placebo (0 mg) or NTX (50 mg) was administered 15 minutes before intranasal methamphetamine (30 mg/70kg). Participants completed two sequential days of testing for each dose combination: On Day 1 (Sample Session) participants received the drug combination and for the following 3 hours, subjective and physiological effects were measured. On Day 2 (Choice Session), participants completed an operant self-administration task in which they could choose between an additional dose of the Day 1 intranasal drug and a monetary reinforcer ($25). Primary outcome measures were peak positive subjective effects (e.g., drug â€œLikingâ€•) from the Sample Session, and the percentage of drug choices from the Choice Session. Complete data sets were available from 13 males and 1 transgender participant (age: 33.4 yrs). Because of an exploratory pharmacogenetic aim (not presented), only participants who self-identified as Caucasian or white were recruited. Participants were primarily users of amphetamine-type stimulants (N=9) or cocaine (N=5), and all had recent experience with faster administration routes (i.e., intravenous, intranasal, or smoked). Methamphetamine significantly increased subjective ratings of drug â€œLiking,â€• â€œGood Effect,â€• and â€œHighâ€• from baseline (p's<0.01). However, methamphetamine effects did not significantly vary as a function of placebo or NTX pretreatment. Similarly, the percentage of methamphetamine (vs money) choices did not vary between the placebo (39.8%) and NTX (39.5%) pre-treatment conditions. The current study failed to find evidence that acute oral NTX alters the abuse potential of intranasal methamphetamine and does not support the role of oral NTX in the treatment of methamphetamine use disorder. Supported by R21DA040225 to JDJ.

Subcortical control of behavior by direct social contact.

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We previously identified the posterior intralaminar thalamic nucleus (PIL) as a relay station of socially relevant sensory information innervating and activating oxytocin-secreting neurons upon social encounter. Here, we addressed to characterize the exact role of the PIL in the regulation of the social behavior, especially its neurons projecting to the preoptic area of the hypothalamus. Projections from the PIL were analyzed using anterograde tract-tracing. We determined the effect of chemogenetic stimulation of the PIL neurons on the social interactions between familiar adult female rats using the DREADD technique. The brain activation patterns were determined following direct social interaction, and also with the exclusion of physical interaction using the c-Fos technique. The selective chemogenetic stimulation of the preoptic area-projecting PIL neurons was performed using double viral injections and also by using intracerebral cannula for CNO administration directly into the preoptic area. PIL projects to several socially implicated brain regions, such as the lateral septal nucleus, the medial amygdala, the medial preoptic area, the paraventricular and dorsomedial hypothalamic nuclei and the infralimbic cortex. Chemogenetic stimulation of the PIL resulted in the activation of the previously anatomically identified target areas and also increased the duration of direct interactions during social behavior. Direct contact during social interaction caused the largest increase in the activity in the medial preoptic area. Specific chemogenetic stimulation of the PIL-preoptic pathway led to elevated direct social contact. The results suggest that posterior thalamic PIL neurons convey socially relevant information to a variety of different forebrain centers, among which the preoptic area is involved in the processing of physical contact. Support: New National Excellence Program of the Ministry of Human Capacities, Excellence Program of the Semmelweis University, NKFIH-4300-1/2017-NKP_17 and OTKA K116538.

The ventral hippocampal CA1, CA3, and medial prefrontal cortex are differentially activated during cue-elicited approach-avoidance conflict resolution.

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An approach-avoidance conflict (AAC) arises when an organism encounters a stimulus that is imbued with both positive and negative valences. The organism must decide to approach or avoid this stimulus by considering the costs and benefits of either behavioural output. We have previously shown, through gamma-aminobutyric acid (GABA) receptor-mediated inactivation and optogenetic archaerhodopsin (ArchT) inactivation in rodents, that ventral CA1 (vCA1) inactivation increases avoidance of a cued conflict scenario, whereas ventral CA3 (vCA3) inactivation facilitates approach towards the conflict scenario. However, the exact timing of hippocampal subfield recruitment during decision making under AAC remains unknown. Additionally, the contributions of neural substrates downstream of the vCA1 or vCA3 in AAC resolution have yet to be elucidated. In the present study, fiber photometry was used to record neuronal activity within the vCA1, vCA3, and medial prefrontal cortex (mPFC) during cue-elicited approach and avoidance conflict and non-conflict behaviours in a Pavlovian Y-maze task. Increases in activity were observed in the vCA1 specifically during the entry into an arm imbued with conflict cues (simultaneous presentation of cues associated with appetitive sucrose and aversive shock outcomes), whereas increases in neuronal activity were observed in the vCA3 during entries into an alternative arm with neutral cues (associated with no outcome). In contrast, increases in neuronal activity were observed in the mPFC during entries into arms imbued with appetitive cues only (sucrose-associated), aversive cues only (shock-associated), as well as conflict cues. A rise in neuronal activity was also seen with sucrose delivery in the presence of both congruent appetitive cues and incongruent neutral cues, but not with shock presentation. These results suggest that the vCA1 and vCA3 are specifically activated when an animal makes the decision to approach or avoid the conflict scenario, respectively. Additionally, these findings implicate the mPFC in cue appraisal in both conflict and non-conflict scenarios, as well as reward expectancy and delivery. This work was supported by CIHR and NSERC.

Gastrointestinal vagal afferents mediate the feeding-induced modulation of anxiety-like behavior

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Gut-brain communication influences far more than just ingestive behavior. Recent data suggest that the feeding status modulates anxiety-like behavior in rats. Decreased anxiety-like behavior in periods of hunger may facilitate food seeking, whereas increased anxiety-like behavior after eating may prevent unnecessary risk-taking. The underlying mechanisms allowing this food-dependent modulation of anxiety-like behavior are poorly understood. Here, we propose that vagal afferent neurons terminating into the gastrointestinal tract (GI VAN) are necessary and sufficient for the modulation of anxiety-like behavior by feeding. To evaluate this, we created three rat models of specific manipulation of GI VAN activity and submitted them to complementary tests of anxiety-like behavior: Elevated Plus Maze, Open Field, Food-induced Neophobia, Acoustic Startle Response. Chemogenetic activation of GI VAN increased anxiety-like behavior independent of the feeding status, indicating that GI VAN activation is sufficient to induce anxiety-like behavior. In contrast, specific chemogenetic inhibition of gastrointestinal vagal afferents reduced anxiety-like behavior of fed rats to the level of fasted rats, indicating that GI VAN are necessary for the anxiogenic of feeding. Moreover, a chronic lesion of GI VAN using CCK-saporin reduced anxiety-like behavior. RNA sequencing of brain areas receiving synaptic inputs from GI VAN indicated that a lesion of GI VAN is associated with transcriptomic changes relevant to anxiety-like behavior. Together, current results indicate a key role for the vagus nerve in the modulation of anxiety and uncover a predominant role for GI VAN in this process. They also bring forth an intriguing idea that disrupted vagal signalling in obesity can contribute to the development of comorbid anxiety disorders. Funding: Wallenberg Center for Molecular and Translational Medicine, Swedish Research Council, Ragnar Soderberg Foundation, Swiss National Science Foundation

Sex differences in guiding fear and reward-seeking behaviors via safety cues.

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The presence of reward, danger and safety cues guide our daily behaviors and aids in emotional regulation. Despite comorbidity of maladaptive fear disorders (PTSD, anxiety) with addiction, there are comparatively few studies investigating discrimination among reward, fear and safety cues in both males and females. Here, we investigated the potential of a learned safety cue to modulate fear as well as reward-seeking behaviors in male and female Long Evans rats. In the first experiment rats were trained to discriminate amongst cues signifying footshock (fear), sucrose delivery (reward) and no outcome (safety and fear+safety compound cues). Male, but not female, rats expressed conditioned inhibition of fear to the compound fear+safety cue during both training and testing. During testing, both male and female rats suppressed reward seeking responses to a newly introduced compound reward+safety cue. Females suppressed reward seeking at twice the rate of males, leading us to hypothesize higher-order conditioning potentially occurred to the safety cue during training when it was presented concurrently with the fear cue. In order to remove the possibility of higher-order conditioning, we then trained rats to discriminate amongst cues signifying footshock (fear), sucrose delivery (reward) and no outcome (safety), before examining conditioned inhibition of either fear or reward-seeking to fear+safety or reward+safety compound cues. Similar to the first experiment, males expressed conditioned inhibition to both the fear+safety as well as reward+safety cues, indicating that a learned safety cue can result in conditioned inhibition of both fear and reward-seeking behaviors. Funding support provided by NIMH R01 Research Grant (R01MH110425)

New players in the brain circuits of movement: A collateral direct pathway from the striatum to the GPe supports motor control.

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The striatum is the main output region of the basal ganglia and a well-established brain region regulating motor behavior. Its neuronal projections are divided into two routes: a direct (D1) and an indirect pathway (D2), classically depicted as having opposite effects on movement and distinct target regions (midbrain vs. globus pallidus=GPe). Interestingly, several anatomical studies have described the existence of axonal collaterals (â€œbridging collateralsâ€•): neuronal bridges arising from D1 neurons yet contacting the target region of D2 neurons: the GPe. These bridging collaterals are plastic in the adult animal and regulated by D2Rs, neuronal activity and motor training. These observations, though indirect, suggest an important role of bridging collaterals in regulating motor function. Their relevance for behavior is however unknown. Here, we used a combination of genetic targeting, in vivo calcium imaging, chemogenetic/optogenetic manipulations and deep learning-based behavioral tracking (DeepLabCut) to determine the role of D1 bridging collaterals in motor function in mice. We found that bridging collaterals were activated during specific movements in a rotarod task. Bridging collateral inhibition also decreased locomotion and impaired rotarod performance. Recent physiology work shows that bridging collaterals preferentially target â€œstop neuronsâ€• expressing Npas1 in the GPe. We here found that stimulation of D1 neurons inhibited native Npas1 motor signals in awake behaving mice. Finally, we found that Npas1 stimulation recapitulated the effects of bridging collateral inhibition by decreasing locomotion and rotarod function. We propose a model by which bridging collaterals support motor function by inhibiting Npas1 neurons in the GPe. Thus, D1 terminals in the GPe act in concert with the canonical D1 terminals in the midbrain by inhibiting a â€œstopâ€• signal going back to the striatum. Funding: NIH, SNSF

Distinct neuronal ensembles within the central nucleus of the amygdala regulate extinction learning.

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Correlational data from histochemical and physiological studies suggest that the central nucleus of the amygdala (CN) is involved in learning when expected events are omitted. Attempts at delineating the causal contribution of CN neurons to this learning have targeted the entire nucleus indiscriminately, disrupting the function of neurons. Recent research using selective approaches have uncovered that not all neurons within a brain area are recruited during learning. Rather, a specific neuronal ensemble supports learning with distinct subsets of neurons likely having different functional roles. We sought to determine the functional role of CN neurons that have been explicitly activated by the omission of an expected reward. c-Fos is a widely used marker for neuronal activity and here, we used the Daun02 inactivation procedure to assess the causal role of activated c-fos-expressing CN neurons in updating reward expectations during extinction. In the present study, male fos-lacZ transgenic rats were trained to expect the delivery of a food reward upon the presentation of an auditory cue. Subsequently, rats received non-reinforced exposure to the reward-associated cue to generate conditions of reward omission, that is extinction, and examine the effect of this on learning. Cell inactivation with Daun02 took place ninety minutes following the start of the non-reinforced session, presumably when the neurons that detected the reward omission were activated and the corresponding c-fos levels were at peak. This led to disruption in behaviour indicative of impaired retrieval of the extinction memory compared to rats that received a vehicle infusion, which left those neurons intact. Additional data show that further extinction learning was retarded in the absence of the neuronal ensemble in the CN. Moreover, inactivating these extinction-responsive CN neurons resulted in greater spontaneous recovery and reinstatement. Lastly, this disruption in behaviour was specific to the CN and not due to drug diffusion into the basolateral amygdala. This work was supported by a Concordia University Horizon post-doctoral fellowship (to BPPL); a FRQS post-doctoral fellowship (to BPPL); a FRQNT Nouveaux Chercheurs grant (to MDI); a NARSAD Young Investigator grant (to MDI); a CIHR Project Grant (to MDI); and the Canada Research Chairs program (to MDI);

Identification of facial markers accounting for expressions associated with gustatory and tactile stimulations in mice.

Olivia Le Moëne1,2, Fatima Twam2,3, Paige Windmill2,4, Max Larsson1. 1, Div of Neurobiology, Deptt of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden. 2, Center for Social and Affective Neuroscience, Dept of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden. 3, Birzeit University, Birzeit, Palestine. 4, College of Medicine and Health, University of Exeter, Exeter, UK. In the last decade, attention has been brought to emotions conveyed by animal facial expressions. Characterizing and interpreting such expressions is important to extract affective states induced by different procedures. However, identifying them remains a major challenge in animal models. In the mouse, previous studies have successfully identified facial expressions associated with different positive and negative situations. Yet, the current methods developed do not offer variables that are both visualizable by a human observer, and finely quantifiable for statistical purpose. To give insight into the processes underlying changes in facial expressions, we identified several points on mice profiles accounting for eye, ear, mouth and snout position and direction, and monitored their relative position on the face during a sequence of stimulations. Following a 5 min baseline, we exposed naÃ¯ve or previously handled c57Bl6j mice to 3 stimulations for 5 min each: a 20 % sucrose solution, a bitter solution (sucrose octaacetate + denatonium benzoate) and gentle brushing by the experimenter's finger. Pictures of mice profiles were extracted from video recordings of the experiment, and previously defined facial parameters measured. Preliminary results showed that compared to baseline, sucrose tasting was associated with ears pointing forward and facing front, while bitter tasting was associated with squinting. Facial expression associated with brushing showed the most marked pattern, including widened eyes, ears pointing backward and facing side. This pattern was even more pronounced during brushing in mice habituated to handling. We found little variation in snout inclination along the stimulations. The facial parameters most affected were eye opening, ear pointing direction, as well as ear position. Different stimulations differentially affected facial features. Funding: LiU, Knut and Alice Wallenberg Foundation.

Interactions between the effects of social group and ethanol on zebrafish behavior.

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Zebrafish (Danio rerio) have been extensively studied in behavioral pharmacology research because they are a promising translational model for humans. Zebrafish share many common genetic and pharmacological mechanisms with humans, making them a potentially useful model organism for human disorders such as drug addiction, alcoholism, and neurodegenerative disorders. Zebrafish naturally exhibit shoaling behavior, and show a strong preference for social stimuli, so the most ecologically relevant results are obtained from zebrafish housed in groups. Nevertheless, experimental constraints â€“ such as the need to identify individuals or expose individuals to specific treatments over time â€“ often require that zebrafish be housed individually or in pairs. Additionally, even when fish are group-housed, they are often tested individually in order to more accurately measure their behavior. It is not currently known how social conditions of housing and testing may affect their responses to experimental treatments, such as ethanol exposure. In the current experiment, zebrafish were housed in groups of four, pairs, or in isolation. They were then exposed to 0.0%, 0.5%, or 1.0% ethanol, and tested in a split-tank diving test. Some animals were tested individually, while others were tested in their housing groups. Our results indicate that the effects of ethanol are significantly influenced by housing status, and make it clear that this variable must be considered when designing experiments and interpreting results of behavioral pharmacological studies in zebrafish.

Role of vasopressin signaling in the ventral pallidum in the sex-specific regulation of social play behavior in juvenile rats.

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The ventral pallidum (VP) is implicated in the regulation of adult social behaviors, such as pair-bonding and sociosexual motivation. However, the role of the VP in regulating juvenile social behaviors, such as social play, is unknown. Social play is predominantly displayed by juveniles of many mammalian species and engagement in social play helps develop social competence. In this study, we first determined whether the VP is involved in regulating social play expression in juvenile rats by temporarily inactivating the VP via bilateral infusions of muscimol, a GABAA receptor agonist. Muscimol treatment decreased social play duration in males and females compared to same-sex control groups. Next, we determined whether exposure to social play altered overall activation of the VP (using fos as a marker), and found that exposure to social play enhanced the number of fos+ cells in males only. We then focused on the vasopressin (AVP) system in the VP as a potential modulator of social play. We examined the structure of the AVP system in the VP in juvenile rats and found sex differences, with denser AVP-immunoreactive fibers and vasopressin 1a receptor (V1aR) binding in males compared to females, but a greater number of v1aR+ cells in females compared to males. Next, we determined whether exposure to social play changed activation of v1aR+ cells in male and female rats. Exposure to social play enhanced the number of v1aR+ cells co-expressing fos in males only. Finally, we determined the causal involvement of AVP signaling in the VP in social play behavior by infusing a specific V1aR antagonist into the VP prior to social play exposure. V1aR blockade in the VP increased social play duration in males but decreased social play duration in females compared to same-sex control groups. Together, these findings are the first to implicate the VP in the regulation of a social behavior in juveniles and suggest that structural and functional sex differences in the VP-AVP system are associated with the sex-specific regulation of social play behavior. This work was supported by the NIH (R01MH102456 to AHV) and the NSF (IOS 1735934 to AHV; DGE-1848730 to JDAL).

Cell-type specific FGF13 regulation of cortical function.

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Fibroblast growth factor homologous factor 13 (FGF13) is a non-canonical member of the fibroblast growth factor (FGF) superfamily that is not a secreted growth factor and functions intracellularly. Long-studied for its role in regulating voltage-gated sodium channels in brain and heart, the full complement of FGF13 molecular functions is undefined. Clinical studies revealed that patients with disruptions or mutations in the Fgf13 gene have early onset cognitive impairment and febrile seizures. FGF13 also has two major alternatively-spliced isoforms in the cortex, one which is predominantly expressed in excitatory and the other in inhibitory cells. To study cell-type specific FGF13 expression in the cerebral cortex, I generated mice lacking FGF13 throughout CamkIIï• ¡-expressing glutamatergic or Gad2-expressing GABAergic interneurons. Mice heterozygous for Fgf13 in interneurons suffer epileptic seizures, consistent with the human phenotype, and hemizygous knockout mice die perinatally. Mice with Fgf13 deficiency in glutamatergic cells exhibit behavioral abnormalities but survive through adulthood. To further define the functional roles of FGF13, I conducted a battery of behavior assays to identify differences between knockout mice and wildtype littermates. My findings reveal distinct roles of Fgf13 in the two cell types, likely mediated by distinct molecular mechanisms. These data show that differential regulation of Fgf13 via alternative splicing generates distinct proteins with different neuronal functions. This work was supported by T32 DA03980 (SL), R01 MH118934 (GSP), and FCLC Dean's Undergraduate Research Grant (ID).

Oxytocin and social buffering: the effects of social-housing and environmental cage conditions on anxiety-like behaviour in the rat.

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Social housing is known to attenuate rodents' stress responses, a well-validated effect termed social buffering. Oxytocin (OT) is strongly implicated in social behaviours and has stress-reducing properties, suggesting that OT may mediate social buffering effects. In recent years many research facilities have adopted the use of individually ventilated cages (IVCs), despite initial evidence suggesting that IVCs create a stressful home-cage environment. One IVC condition that may be responsible for increased stress levels is the pressurized ventilation system. IVCs can be operated under negative or positive pressure, however, little is known about how air pressure might influence behaviour. It is also unknown if social buffering effects carry-over to rodents housed in IVCs, and whether the OT system is implicated. Thus, the current study examined the stress-protective effects of pair-housing in rats housed under different IVC air pressure conditions, and the potential involvement of the OT system. Rats were assigned to single- or paired-housing in IVCs under negative or positive pressure. After four weeks, their anxiety-like behaviour was measured in the novelty-induced suppression of feeding test (NSFT), the elevated plus-maze (EPM), and the shock-probe burying test (SPBT). Following behavioural testing, their brain tissue was processed to examine oxytocin-immunoreactivity (OT-ir) in the paraventricular nucleus (PVN) of the hypothalamus. Results indicated a test-specific effect of social buffering, as pair-housing reduced anxiety-like behaviour in the SPBT but had no effects in the NSFT. In the EPM, social buffering was only evident in rats housed under positive pressure. Preliminary results from the OT-ir analysis revealed no differences in the number of OT-ir cells between single- and pair-housed animals. However, there was a significant positive correlation between the number of OT-ir cells in the magnocellular region of the PVN and behaviour in the EPM. Given the increased use of IVCs in rodent research facilities, understanding how cage-conditions might influence rodent stress levels is imperative to future research. Research supported by the Natural Sciences and Engineering Research Council of Canada.

Encoding of distinct OCD-relevant behavioural disturbances in orbitofrontal cortex neurons projecting to dorsal striatum.

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Neuroimaging studies implicate dysfunction in orbitofrontal cortex (OFC) to striatum circuits in obsessive compulsive disorder (OCD). In OFC, hyperactivity is observed during symptom provocation, and impaired OFC recruitment observed during tasks probing perseverative decision-making including reversal learning. In vivo single-cell imaging in the Sapap3 knockout (KO) mouse model, which displays OCD-relevant perseverative grooming and impaired reversal learning, can be used to determine how these behaviours are encoded in OFC broadly, and selectively in OFC neurons projecting to striatum. Male and female mice were injected in lateral OFC (lOFC) with virus encoding fluorescent calcium indicator under a general promotor (hsyn-GCamP6f) or cre-dependent promotor (DIO-GCamP6f) combined with injection of retrograde-cre virus in dorsal striatum. Gradient-index (GRIN) lens was implanted above lOFC to visualize neural activity using Inscopix miniscopes (n=12KO/8 wildtype (WT) littermate controls, ~5 months of age). Calcium imaging was performed during grooming assessment and reversal learning, and aligned to behaviors of interest (correct/incorrect responses, rewards, initiation/termination of grooming). Time spent engaging in compulsive grooming varied across individual KO mice (7-70% of time spent grooming). When assessing all OFC neurons (hsyn-GCamP6f) Sapap3-KO mice had significantly more inhibited OFC neurons at the onset of grooming relative to WT, and % inhibited cells was correlated with more severe compulsive grooming phenotype in KOs. During reversal learning, reward responsive neurons showed increased magnitude of neural activity in KO compared to WT. When assessing OFC neurons that projected to the dorsal striatum (retrograde cre mediated GCaMP6f expression), this recapitulated the findings related to reward-responsive neurons from hsyn-GCaMP6f studies, but not findings related to grooming. These data suggest that Sapap3-KOs show distinct patterns of lOFC activity change associated with severity of perseverative grooming versus reversal learning. Cells projecting to dorsal striatum appear to mediate changes associated with reversal learning, whereas grooming may be mediated by other projection targets. Funding: R21 MH116330

Alcohol drinking patterns and compulsive use in an automated home-cage design for rats.

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In humans, a major characteristic of alcohol use disorder (AUD) is alcohol use despite negative consequences. In our lab, we use an operant behavioural paradigm to model alcohol use despite negative consequences in rats. Rats are first trained to self-administer alcohol in operant chambers, and then self-administration is paired with mild footshock punishment. Rats that continue to drink alcohol despite negative consequences are designated punishment-resistant users. As with the majority of alcohol research in rodents, our paradigms are restricted to short-access traditional operant chamber training, which limits data collection possibilities and may not be the most representative to the human experience. Humans tend to have alcohol access throughout the day, and often those with AUD drink at different times of day compared to others who drink. Our previous research suggests that the amount of alcohol taken during self-administration does not predict subsequent sensitivity to punishment. However, these comparisons were limited to short access self-administration in operant chambers. To address these limitations, we have piloted the use of a combined home-cage and operant-chamber for alcohol self-administration, connected by a tunnel. 24 rats (12m,12f) were housed and trained in the alcohol self-administration combi-cages, with ad-lib access to food and water. Animals first underwent 24 days of 24hr on/off intermittent access to alcohol, to compare combi-cage alcohol consumption with traditional home-cage bottle access. Next, we shortened access to 3hrs on/off and finally 30mins on/off to determine how rats change their drinking patterns due to limited access. Finally, we paired alcohol self-administration with mild footshock punishment, to identify punishment-resistant rats. Our aim here was to identify if individual differences in alcohol drinking patterns across time could predict vulnerability to punishment-resistant alcohol drinking. We also identified sex differences in alcohol drinking patterns, which differed from those observed in traditional limited-access operant training. Combi-cage alcohol-self administration allows us to collect temporal alcohol consumption data and identify their relationship to the development of addiction-like behaviour. Funding: NWO.

S-equol mitigates motivational deficits and dysregulation associated with HIV-1.

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HIV-1 seropositive individuals exhibit an increased neurobehavioral burden, characterized by a greater prevalence of motivational deficits (i.e., apathy) and dysregulation (i.e., addiction) relative to their seronegative counterparts; motivational alterations which are associated with profound functional consequences. The persistence of motivational alterations in HIV-1 seropositive individuals, despite treatment with combination antiretroviral therapy, necessitates the development of innovative adjunctive therapeutics. S-Equol (SE), a selective estrogen receptor beta agonist, has been implicated as a neuroprotective and/or neurorestorative therapeutic for HIV-1 associated neurocognitive disorders; its therapeutic utility for motivational alterations, however, has yet to be systematically evaluated. Thus, ovariectomized Fischer (F344/N) HIV-1 transgenic (Tg) and control rats received either an oral dose of 0.2 mg SE (Control: n=11; HIV-1 Tg: n=11) or vehicle (Control: n=10; HIV-1 Tg: n=10). Operant conditioning procedures were utilized to evaluate goal-directed and drug-seeking behaviors. First, at the genotypic level (HIV-1 Tg Vehicle vs. Control Vehicle), motivational deficits in HIV-1 Tg rats were characterized by a diminished reinforcing efficacy of, and sensitivity to, sucrose. Motivational dysregulation was evidenced by enhanced drug-seeking for cocaine in HIV-1 Tg rats relative to controls. Second, in HIV-1 Tg animals, treatment with SE (HIV-1 Tg Vehicle vs. HIV-1 Tg SE) ameliorated both motivational deficits and dysregulation. Third, following a history of cocaine self-administration, HIV-1 Tg animals treated with vehicle exhibited lower levels of dendritic branching and a shift towards a more immature dendritic spine phenotype in medium spiny neurons from the nucleus accumbens. Treatment with SE, however, led to long-term enhancements in dendritic spine morphology in HIV-1 Tg animals supporting a potential underlying basis by which SE exerts its therapeutic effects. Taken together, SE restored motivated behavior in the HIV-1 Tg rat, expanding the potential clinical utility of SE to include both neurocognitive and affective alterations. Funding was provided by: NIH Grants DA013137, HD043680, MH106392, NS100624.

Working and reference memory across the lifespan of male and female F344 rats.

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Women typically live to older ages than men and are more likely to be afflicted by Alzheimer's disease (AD), an age-associated brain disorder characterized by multi-dimensional cognitive decline. However, few studies have robustly evaluated how biological sex influences trajectory of cognitive changes in preclinical models of aging. Indeed, fundamental studies of memory loss in normally aging rats could reveal the basis for sex-related susceptibility to AD or worse cognitive symptoms. With this question in mind, our lab conducted a cross-sectional study of cognitive differences in male and female F344 rats at 4 (young adult), 12 (middle-aged) or 22 (aged) months of age. Rats were characterized using an operant delayed-match-to-sample (DMTS) test of working memory and in the Morris water maze (MWM) on a spatial, place-learning test of reference memory. In the DMTS task, we observed a significant effect of age on working memory choice accuracy but no reliable differences between males and females or interaction between sex and age. Post hoc tests determined that aged rats were less accurate than young adults or middle-aged rats while these latter two age groups were not reliably different from each other. In the MWM task, analysis of probe trials distributed across days of testing determined that training interacted with age and sex. To examine this interaction, we calculated the weighted sum of probe trial performance, and found that, overall, males exhibited better spatial learning than females and that middle-aged and aged rats were impaired relative to young adults. We conclude that spatial reference memory is more sensitive to early decline with age than working memory and that spatial reference memory of females is less accurate than in males. These differences suggest that focused study of the sex-specific neurobiological underpinnings of the aging hippocampus could lead to a better understanding of the fundamental mechanisms that contribute to increased severity of memory problems in older women. Funding Acknowledgements: South Carolina Honors College and NIH Grants K01AG061263, P20GM109091, P20GM103641.

Characterizing ultrasonic vocalization in a gene-environment interaction model for autism

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Autism spectrum disorders (ASDs) are complex neurodevelopmental disorders estimated to affect 1 in 68 children. Core symptoms of ASD include deficits in social communication and language development. Of the many risk factors for ASD, the Contactin associated protein-like2 (Cntnap2) gene is known to be important for language development in humans. However, no single risk factor can fully explain the ASD prevalence and pathophysiology. The 'double-hit hypothesis'• proposes that ASD is often a combined result of genetic mutations and environmental challenges, and that different risk factors likely affect common neurodevelopmental pathways leading to the core symptoms of ASD. The present study utilized the framework of the 'double-hit hypothesis'• to investigate potential compounding effects of ASD risk gene mutation, breeding conditions, or maternal immune activation (MIA) during pregnancy on vocal communication.Maternal isolation-induced ultrasonic vocalizations (USVs) were recorded from Cntnap2 wildtype (WT), heterozygous (Het), and homozygous knockout (KO) rat pups at selected postnatal days before weaning. In the first experiment, KO rat pups originated from either heterozygous or homozygous breeding pairs. In the second experiment, we compared USVs from heterozygously bred pups exposed to poly(I:C) MIA or vehicle during pregnancy. We used the DeepSqueak software suite (Coffey et al., 2019) to train an artificial neural network for automated USV call detection and classification, and analyzed acoustic call characteristics as well as more complex USV features such as call sequences. Comparison of USVs suggests an interaction between homozygous loss-of-function mutation in the Cntnap2 gene and environmental factors, exacerbating alterations in call parameters such as principal frequency of calls, but not others such as total number of calls and call duration. The results will be discussed in the context of the neurobehavioral origin of ASD-related USV deficits and contribution of genetic and environmental challenges to vocal communication in terms of the 'double-hit hypothesis'• .This study was supported by CIHR, NSERC, and the Deutsche Forschungsgemeinschaft (DFG) - 442662585.

RMTg and NAc Afferents to Ventral Tegmental Area in Opioid Withdrawal-Induced Aversion

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Aversion is induced by withdrawal from drugs of abuse and is thought to be a critical component in the cycle of addiction. While the initial stages of drug use might be motivated by feelings of euphoria, compulsive drug use is motivated by a desire to avoid the negative emotional effects of withdrawal. Critically, the neurobiological mechanisms of opioid withdrawal-induced aversion are still being fully elucidated. Dopamine (DA) neurons in the ventral tegmental area (VTA) have been studied extensively and are known to be involved in the rewarding properties of opioid use, but less is known about the role they play in expressions of withdrawal-induced aversion. Even less is known about the afferents that affect them. This project used chemogenetics to inhibit two GABAergic VTA afferents previously shown to be involved in aversion (the rostromedial tegmental nucleus (RMTg) and nucleus accumbens (NAc)), during morphine withdrawal-induced conditioned place aversion (CPA).Methods: C57BL/6J mice received pENN.AAVrg.hSyn.HI.eGFP-Cre.WPRE.SV40 in the VTA. Experimental mice received pAAV-hSyn-DIO-hM4D(Gi)-mCherry in the RMTg or NAc shell; controls received pAAV-hSyn-DIO-eGFP. Mice were subjected to withdrawal CPA using spatial preference boxes with two distinct sides and a neutral zone. During conditioning, mice received a cocktail of morphine (10 mg/kg) and clozapine-n-oxide (CNO, 1.0 mg/kg) 30 minutes before being placed in the conditioning box. Immediately before placement in the box, mice received naloxone (2.5 mg/kg) and were confined to one side. On withdrawal-free sessions, mice again received CNO 30 minutes before confinement but received saline as the second injection. Analysis compared time spent on the withdrawal-paired side before and after conditioning.Results: Preliminary results suggest that RMTg inhibition reduced CPA compared to controls. NAc inhibition had no effect. These results suggest that GABAergic inputs to the VTA from the RMTg may participate in opioid withdrawal-induced aversion, although further testing will be needed to verify this. Reducing the severity of aversion during withdrawal from drugs of abuse, perhaps through inhibition of the RMTg, could be a useful therapeutic intervention to reduce reinstatement of drug-seeking.

Discriminative fear/safety conditioning identifies different endophenotypes of stress-relevant learning performance in mice and rats.

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In humans, impaired inhibition of fear upon presentation of a safety cue is associated with the risk to develop PTSD after a traumatic and stressful experience. Investigating individual differences in fear inhibition in rodents could thus unravel the neurobiological basis of stress resilience and vulnerability. We therefore investigated individual differences in fear inhibition performance using a newly developed discriminative fear/safety conditioning paradigm in mice and a recently established discriminative fear/safety/reward conditioning procedure in rats. In the mouse paradigm we identified three qualitatively different subgroups of animals. Good safety learners presented high fear in the fear- and low fear in the safety condition, as well as good discrimination for the fear cue compared to the background context and the safety cue already early on in conditioning. Poor safety learners showed equally high freezing levels in the fear and the safety condition, while non-learners showed equally low levels to both. In the rat paradigm good and poor safety learning subgroups differed only in the extent of performance, but both groups were able to reduce their fear response upon co-presentation of the safety cue with the fear cue. However early during discriminative conditioning the later poor safety learners showed transiently better cue discrimination than the later good safety learners. Together, we identified different endophenotypes of stress-relevant learning performance even without stress presentation. Fear inhibition may be related to the risk for PTSD development and could be used to identify a priori existing differences in resilience and vulnerability. Funding: Feodor-Lynen fellowship to IM, NIMHR01-MH110425 to SS, German Research Foundation (STO 488/6) to OS.

Geometry of abstract learned knowledge in the hippocampus.

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Hippocampal neurons encode physical variables such as space or auditory frequency in cognitive maps. In addition, human fMRI studies have shown that the hippocampus can also encode more abstract, learned variables. However, their integration into existing neural representations of physical variables is unknown. Using 2-photon calcium imaging, we show that individual dorsal CA1 neurons jointly encode accumulated evidence with spatial position in mice performing a decision-making task in virtual reality. Nonlinear dimensionality reduction estimated the neural manifold to be ~4-6 dimensional. Within this low-dimensional space, both physical and abstract variables were jointly mapped in an orderly fashion, creating a geometric representation that we demonstrate to be similar across animals. The existence of conjoined cognitive maps suggests that the hippocampus performs a general computation â€“ to create geometric representations of learned knowledge instantiated by task-specific low-dimensional manifolds.This work was supported by the NIH grants U01NS090541, U19NS104648, and F32MH119749.

Evidence implicating social contact-induced changes in the oxytocin system in the anxiolytic-like effects of social housing in the rat.

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Social contact is known to reduce or "buffer" rodents' responses to acute stress. Although the mechanisms responsible for social buffering remain unclear, the neuropeptide oxytocin (OT) is thought to play a role. OT is produced by the paraventricular nucleus (PVN) and possesses strong anti-stress and anxiety-reducing properties. The production of OT is enhanced by OT release. Given that OT is released in response to tactile stimulation, it might be the case that social housing upregulates OT production. Thus, the goal of the current study was to explore whether social housing-induced changes in rats' defensive behaviours map onto changes in the number of OT immuno-labelled cells in the PVN. Rats were randomly assigned to either single- or paired-housing for five weeks. Then, they were tested in two well established behavioural paradigms modelling anxiety-related responding: the elevated plus maze (EPM) and a modified version of the novelty-induced suppression of feeding test (NSFT). A week later, their brains were harvested and processed using immunohistochemistry for OT-immunoreactivity. Pair-housed rats trended towards a higher percentage of open-arm exploration in the EPM relative to single-housed rats. Additionally, pair-housed rats took significantly less time to initiate consumption of a palatable snack in the NSFT and had significantly more OT-positive cells in the ventral region of the PVN. The number of OT-positive cells in the ventral PVN correlated negatively with their latencies to begin consuming the snack on Day 1 of the NSFT. Overall, these findings suggest that social buffering may be mediated, at least in part, by social contact-induced remodelling of the OT system. Acknowledgements: funded by NSERC.

Sex-dependent effects of antidepressant mirtazapine in offspring from CUS dams

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Depression is a life-threatening form of mental illness that affects women twice more likely than men, especially during pregnancy and breastfeeding (14-23%). The administration of antidepressants in this critical period represents a serious health concern for both the mother and the child, as the consequences of maternal antidepressant treatment on the developing organism are not well known. New generation antidepressants, such mirtazapine, that targets both the serotonergic and noradrenergic systems in the CNS, represent a new focus of research. However, little is known about the safety of these drugs for the developing organism. Our aim was to study the effects of maternal depression and/or antidepressant mirtazapine treatment on the neurobehavioral development of their adolescent offspring. Female Wistar rats were submitted to a 3-week chronic unpredictable stress (CUS) procedure and then were mated. Dams were treated with mirtazapine, administrated orally from day 10 of gestation until day 21 post-partum at a dose of 10mg/kg/day. After weaning, offspring was submitted to behavioral tests: FST and EPM. In males, we observed significant main effect of stress on motor activity (F (1, 30)= 3.93; pâ‰¤0.05) and on the percentage of time spent in the closed arm (F (1, 30)= 4.53; pâ‰¤0.05). We observed marginally significant main effect of stress Ã— mirtazapine interaction on motor activity (F (1, 30)= 3.69; p=0.06) and trend of stress Ã— mirtazapine interaction on the percentage of time spent in the closed arm (F (1, 30)= 3.37; p=0.07). In females, we observed marginally significant effect of mirtazapine (F (1, 32)= 3.70; p=0.06) and trend in effect of stress (F (1, 32)= 3.06; p=0.08) on the percentage of time spent in the open arm in the EPM. The results of our study show that pre-gestational CUS and/or antidepressant treatment with mirtazapine may cause increased anxiety-like behavior in males with decrease in general activity. These changes manifest in long-term neurobehavioral development and sex-dependent manner. The study was supported by the grants VEGA 2/0124/19 and APVV-19-0435.

Prefrontal Circuit Mechanisms Underlying Stress Effects on Effort-Based Decision Making.

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Deficits in effort valuation (EV), a cost-benefit analysis comparing the magnitude of anticipated rewards with required effort, may contribute to anhedonia in stress-related disorders. The prefrontal cortex supports EV however, individual circuits may encode varying aspects of learned reward and effort expectations to modulate behavior. Methods: We developed a novel head-fixed EV task for simultaneous 2-photon calcium imaging of corticostriatal and corticoamygdalar projection neurons. Male and female mice were trained to discriminate reward- and non-reward-predictive auditory cues. Later in training, high-effort trials were introduced, signaled by a tactile cue and increased lickport distance. Lick responses were quantified as anticipatory or consummatory if occurring prior to or following reward delivery, respectively. Mice underwent chronic nondiscriminatory social defeat stress (CNSDS) after acquisition. Each cell was tested for low-dimensional encoding of reward- and effort-predictive cues and the accuracy of reward and effort decoding from population activity was determined at baseline and following stress. Results: Mice show behavioral sensitivity to reward and effort conditions as measured by average baseline-subtracted anticipatory and consummatory licking. Corticostriatal and corticoamygdalar neurons exhibit heterogenous responses to reward- and effort-predictive cues. High-dimensional coding mechanisms show that decoding accuracy is improved on high-effort trials in the corticostriatal population specifically. Furthermore, stress biases behavioral responding towards low-effort reward-seeking in a subset of mice. CNSDS also reduced the accuracy of decoding rewarded trials from corticostriatal population activity during anticipatory and consummatory periods and decoding reward and effort across the whole trial in the corticoamygdalar population. Conclusion: Mice are sensitive to reward value and effort expenditure in our task, and stress-susceptible mice are impaired in high-effort responding. Stress interferes with activity in two important dmPFC pathways. Future studies will determine mechanisms underlying individual variation in stress responsivity. Funding: F32 MH117973 and WCM JumpStart Research Award to PKP.

Ergothioneine - promising therapy for prenatal hypoxia?

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Insufficient supply of oxygen to the fetus (prenatal hypoxia) is one of the impacts capable to disrupt its development. The central nervous system is specifically sensitive to hypoxia, and consecutive increase of free radicals may lead to deterioration of cognitive processes and neuropsychiatric defects later in life. Up to date, no treatment is approved for all hypoxic patients. Ergothioneine (E) is a potent natural antioxidant that may offer broader application even in risk patients. AIM: Our study aimed to assess the use of ergothioneine as potential new therapy for behavioral consequences of prenatal hypoxia. METHODS: Pregnant Wistar rats were subjected to hypoxia (10,5%O2) on gestational day (GD) 20 for 12h. E was administered p.o. 35mg/kg on GD6-21. Postnatal development of pups was assessed by air-righting and startle reflex test. Behavior in adulthood was assessed by open field and novel object recognition test. RESULTS: E normalized the development of startle reflex in hypoxic pups but impaired its development in intact pups (p<0.001). In the air-righting test, E decreased success rate both in hypoxic (p<0.01) and intact pups (p<0.05) compared to controls. In adulthood, E normalized anxiety-like behavior in the open field in hypoxic males but caused anxiety-like behavior when administered to intact males (p<0.05). Hypoxic females treated by E had higher novel object recognition than untreated hypoxic females (p<0.05). Hypoxic males, however, had a lower success rate after treatment compared to untreated hypoxic males (p<0.05) in this test caused by a surprising trend to a higher success rate of untreated hypoxic males compared to controls (p=0,08). CONCLUSION: E showed potential in alleviating some of the negative impacts of prenatal hypoxia on the development and behavior of the rat offspring. However, its effect was not beneficial when administered to intact rats. Future studies will be needed to assess the further potential use of ergothioneine in clinical practice. Supported by VEGA 2/0154/20 and SAS Programme for PhD students grant APP0054.

CRF neurons in a paraventricular thalamic circuit integrate reward- and threat- related information to regulate approach-avoidance conflict

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Animals need to constantly make decisions between approaching food and avoiding potential threats to survive, but which neural circuits regulate this motivational conflict remain largely unknown. We designed an ethologically relevant "food approach vs. predator threat avoidance" conflict test that enabled us to investigate the neural circuits involved in threat and reward integration. In this model, rats were initially trained to press a lever for sucrose in the presence of an audiovisual cue. During the conflict test, a source of predator odor was positioned in the food area adjacent to the lever. Rats exhibited robust defensive behaviors and a clear suppression in food-seeking responses when the food cues were concomitantly presented with predator odor. Using in situ hybridization and in vivo single-unit recordings from photoidentified cell-types, we identified a subpopulation of neurons in the anterior portion of the paraventricular nucleus of the thalamus which express the stress neuropeptide corticotrophin-releasing factor (aPVTCRF) and are preferentially recruited during conflict. Chemogenetic inhibition of aPVTCRF neurons biased rats' behavior towards food seeking, suggesting that these neurons are indispensable for the expression of defensive behaviors and suppression of food-seeking responses observed during conflict. Anterograde viral tracing showed that aPVTCRF neurons project densely to the nucleus accumbens (NAc), and optogenetic activation of the aPVTCRF-NAc pathway recapitulated the food-seeking suppression and avoidance responses induced by predator odor. In addition, we identified the ventromedial hypothalamus (VMH) as a critical input to aPVTCRF neurons, and demonstrated that aPVT-projecting VMH neurons are activated by predator odor and necessary for the expression of defensive responses exclusively during conflict. Together, our findings describe a subpopulation of neurons in a hypothalamic-thalamostriatal circuit that suppresses reward-seeking behavior in the presence of threats.Funding: NIH R00-MH105549, R01-MH120136

Modeling behavioral therapeutic approaches for major depression disorder (MDD) in rats: The impact of effort-based reward (EBR) training on sex-dependent stress responsiveness and emotional resilience.

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The effectiveness of cognitive/behavioral therapies has been well-established, yet appropriate preclinical models for behavioral therapies are rare. Accordingly, the current study employed a rodent effort-based rewards (EBR) model to explore contingency-training (CT) effects on neurobiological markers of emotional resilience. Male and female rats were assigned to either an EBR CT group or a non-contingent (NC) group (n=7 for each group; n=28). Following 6-weeks of training focused on building associations between physical effort (digging) and desired outcomes (food reward), rats were assessed in two behavioral tasks. In the novelty-suppressed eating task, EBR rats consumed less food reward than NC animals (p<.01). In the repeated swim assessment, EBR rats exhibited a shorter latency to float (viewed as adaptive energy conservation) than NC animals (p<0.05); further, females had a shorter latency than males (p<.01). In contrast to previous work, EBR training had no effect on endocrine measures; however, females exhibited a significantly higher DHEA/CORT ratio (p<.01), a physiological marker of emotional resilience. Preliminary PCR analyses targeting Slc6a mRNA levels associated with carrier proteins for several relevant neurochemicals (e.g., 5-HT, GABA) indicate sex-dependent effects with EBR males exhibiting a downregulation in the nucleus accumbens (NAC) and EBR females exhibiting an upregulation compared to NC animals. BDNF involvement assessed via TrkB mRNA levels indicated an EBR effect with males and females exhibiting upregulation in the NAC and a downregulation in the cingulate cortex compared to NC rats. Histological analyses of biomarkers of emotional resilience in hippocampal sections (e.g., DCX- and BDNF-ir) are ongoing. In sum, although heightened vigilance in the EBR group was unexpected in the NSE task, the current results provide further support of the influences of EBR training on emotional adaptability and regulation in the forced swim task along with sex-dependent effects in stress-related/induced neurobiological responses. Funding supported by NIMH 1R15MH117628-01A1 to KGL.

Prenatal exposure to methylazoxymethanol acetate: Effect on pups' ultrasound vocalizations.

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Schizophrenia is typically diagnosed during late adolescence or early adulthood, but subtle deficits in communication and sociability are often evident from early infancy. Although the presence of these deficits is evident and a robust predictor of a poor outcome, early behavioural changes have not been well characterized in a rodent model of schizophrenia. Maternal potentiation of rodent vocalization is a promising model of early life social bonds that can be a useful tool in research. Short interactions with mother just before isolation have been found to increase vocalizations in rodents. Using the neurodevelopmental model of schizophrenia, based on the prenatal injection of methylazoxymethanol acetate (MAM; 22 mg/kg; ED 17), we assessed early communicative behaviour by maternal-separation induced ultrasonic vocalizations (USV) in ten-days old pups. Pups' USV were recorded before and after interactions with mother. The results show that short contact with mother led to the greater number of USVs in control, re-isolated pups. This potentiation was not observed in MAM-treated pups. In addition as compared with controls, MAM pups displayed: i) lower frequency peak, ii) extended call duration and iii) increased percentage of flat sounds. These data demonstrate that MAM-exposed pups show atypical reaction to the maternal separation and potentiation, which can be interpreted as dysfunctional behaviour. This communicative behaviour might be considered as a marker for early assessment of schizophrenia-like behaviour in rodents. This study was supported by the Polish National Science Centre grant NCN 2016/23/B/NZ7/01131.

Striatal dopamine dynamics guide stimulus-response learning

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Strategies for routine behaviours, or habits, provide a rapid, efficient means for decision making, but come with a loss of behavioural flexibility. Many psychiatric and neurodegenerative disorders are characterized by aberrant decision-making and dysfunctional habit formation, including addiction and obsessive-compulsive disorder. Striatal neurocircuitry underlies the habitual control of behaviour by facilitating synaptic plasticity and strengthening stimulus-response (S-R) associations. One essential neurotransmitter that regulates activity within the striatum is dopamine, and a loss of modulatory control of striatal dopamine has been shown to impact the rate of habit formation and associated processes. However, little is known about how S-R learning is supported by fast changes in extracellular dopamine levels across different striatal subregions. Here, we uniquely combined automated touchscreen cognitive assessments, fibre photometry, and the recently developed genetically-encoded dopamine biosensor, GRABDA, to record in vivo dopamine dynamics across the dorsomedial striatum, dorsolateral striatum and nucleus accumbens while mice performed the Visuomotor Conditional Learning Task - an established cognitive task that assesses S-R learning. We show that dopamine responds dynamically across task events, and that these response patterns differ topographically across the striatum. Together, these findings suggest that the dopamine system in different striatal subregions plays distinct, but complementary, roles in stimulus-response learning.

Milnacipran ameliorates executive function impairments following frontal lobe TBI in rats

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Traumatic brain injuries (TBIs) affect over 10 million patients annually worldwide, causing long-term complex cognitive and psychosocial impairments. Frontal lobe TBIs commonly impair executive function, but lab TBI models typically study spatial learning and memory. We evaluated clinically-relevant cognitive-behavioral assessments sensitive to frontal lobe TBI in rats and assessed benefits of the serotonin-norepinephrine reuptake inhibitor, milnacipran (MLN). Two attentional set shifting tasks (AST) evaluated rats' sustained attention and cognitive flexibility through their ability to locate food-based rewards by learning, unlearning, and relearning changing rule sets with shifting salient cues. We hypothesized MLN treatment would improve post-TBI performance. Adult male rats, pretrained for 3-4 weeks to reach stable pre-injury operant performance, were isoflurane-anesthetized, subjected to a unilateral prefrontal cortex controlled cortical impact (2.4 mm deformation depth at 4m/s) or sham injury, and randomized as: TBI-vehicle, TBI-MLN, and respective sham controls. Milnacipran or vehicle was administered intraperitoneally via implanted osmotic minipumps (continuous infusions: MLN 30 mg/kg/day or normal saline 60 µL/hr). Rats had a 10 day post-TBI/sham recovery before completing light/location-based operant AST for 10 days and odor/media-based digging AST on the last day (26-27 days post-injury). Data were analyzed with repeated-measures ANOVAs with Fisher's LSD post hoc if appropriate. Both AST tests revealed significant deficits in TBI-vehicle rats, seen as elevated total trials and errors ($p<0.05$), that normalized in MLN-treated rats ($p<0.05$). This first dual AST assessment in the same TBI rats demonstrates AST is sufficiently sensitive and robust to detect subtle attentional and cognitive flexibility executive impairments after frontal lobe TBI in rats. MLN shows promising attenuation of post-TBI executive function deficits, meriting further investigation. Funding: NIH R21NS099683-01 (PI Bondi)

Maternal high-fat diet and neonatal lipopolysaccharide exposures interact to program sex-specific changes to anxiety-related behavior in adult rats

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Both neonatal infections and exposure to maternal obesity are inflammatory stressors in early life that are linked to increased rates of psychopathologies related to anxiety behavior. Epidemiological studies indicate that neonates born to mothers with obesity have a higher likelihood of developing neonatal infections, however effects on offspring behavior resulting from the combination of these stressors have yet to be investigated. Therefore, the aim of this study was to characterize the behavioral phenotype at adulthood resulting from neonatal infection (simulated by bacterial lipopolysaccharide (LPS) injections) in rat offspring born to dams consuming a high-fat diet (HFD). Anxiety-related behavior was assessed at the adult stage with elevated-plus maze and light-dark box testing. Additionally, behaviors in response to adult LPS challenge were assessed in the open-field test. To understand potential mechanisms driving changes in behavior, levels of the stress hormone corticosterone were measured in the plasma, and immunofluorescence of the hippocampus and amygdala, key brain regions involved in regulating anxiety-related behaviors, was also conducted to measure density of microglia, brain resident immune cells that can increase anxiety-related behavior. We found that females exposed to both HFD+LPS exhibited increased anxiety-related behaviors compared to offspring exposed to control diet, control diet+LPS, and HFD alone, whereas the HFD+LPS males exhibited alterations to anxiety-related behavior compared to the other groups. In the dorsal hippocampus and central nucleus of the amygdala, HFD+LPS females displayed elevated numbers of microglia compared to control diet females, while the HFD+LPS males displayed levels similar to the control diet males in most of the regions examined. These findings suggest that exposure to multiple inflammatory stressors in early life alter anxiety-related phenotypes in a sex-specific manner.Funding: Discovery grant from the Natural Sciences and Engineering Council of Canada (NSERC) to Dr. Patrick O. McGowan.

Elucidating the Role of Endocannabinoid Signaling and the Lateral Septum in Regulating Fear Generalization.

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Fear generalization is a conserved survival mechanism that can become maladaptive in the face of particularly traumatic situations, a feature central to certain anxiety disorders such as post-traumatic stress disorder (PTSD). However, the neural circuitry and molecular mechanisms underlying fear generalization remain unclear. Recent studies have increasingly implicated endocannabinoid signaling and the lateral septum as potential therapeutic targets for reversing PSTD-like behaviors. Here, we build on this work by using a behavioral model of fear generalization in mice involving foot shocks during auditory fear conditioning. We find that a mouse model with a single nucleotide polymorphism that ultimately results in increased anandamide expression (FAAH C385A) exhibit accelerated extinction of generalized fear in comparison to wildtype mice. We then also explore the utility of pharmacological tools that target the endocannabinoid system for reducing fear generalization behavior. Additionally, we use fiber photometry to parse out underlying neural activity in the lateral septum during the acquisition and recall of generalized fear, and find intensity-specific patterns of activity during both the initial acquisition and later expression of fear generalization behavior. Overall, these findings indicate a novel role for endocannabinoid signaling and the lateral septum in modulating fear generalization. Funding provided by NIH grant R01NS052819.

Four phenotypes: a novel animal model linking various levels of reinforcement sensitivity with a putative predisposition to specific mood conditions.

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Accumulating experimental evidence and theoretical models implicated various patterns of reinforcement sensitivity and resulting personality traits, in the predisposition, pathoplasticity, and recurrence of affective disorders. Despite this fact, there has been no systematic preclinical research aimed at investigating these interactions in an animal model until now. To address this need, in the present study, we introduce a novel animal model linking various levels of reinforcement sensitivity, measured as stable and enduring traits, with a putative predisposition to various mood conditions. This novel approach became possible thanks to employing a preclinical version of the probabilistic reversal learning (PRL) paradigm, which allows simultaneous analysis of the reactions of animals to positive and negative feedback. The reinforcement sensitivity was tested in 10 consecutive PRL tests. The assessment of the sensitivity to negative reinforcement as a stable and enduring behavioral trait was made based on an average ratio of lever changes following misleading non-reward (probabilistic lose-shifts), while the assessment of the sensitivity to positive reinforcement was made based on an average ratio of pressing the same lever (win-stays) following both true and misleading rewards. The animals with scores above the median were classified as 'sensitive'; while those with scores below the median as 'insensitive'; to a given reinforcement. Based on this 'reinforcement sensitivity screening', each rat was assigned to one of 4 groups representing various reinforcement sensitivity phenotypes: 1) 'irritable' (the rats sensitive to both types of feedback); 2) 'dysthymic' (the rats insensitive to both types of feedback); 3) 'pro-depressive, (the rats sensitive to negative and insensitive to positive feedback), and 4) 'pro-manic' (the rats insensitive to negative and sensitive to positive feedback). The results of validation of this model against various behavioral correlates of depressive symptoms that can be measured in rodents will be discussed in the light of cognitive biomarkers and personalized antidepressant treatment. This work was supported by the Polish National Science Centre (Grant 2016/23/B/NZ4/01562).

Evaluation of Mental Well-Being, and Perceived Stress in Syrian Refugees in Houston, Texas, USA

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Syrians are the largest forcibly displaced population in the world, amounting to 13 million. Nearly 20,000 Syrian refugees have come to the United States (US) since the civil war in Syria began in 2011, and approximately 150 families have resettled in Houston, Texas. Early intervention at multiple fronts is critical to facilitate successful integration of Syrian refugees into the American society and thus relieving socio-economic and health burden on the society. As a first step in this direction, a study was conducted with the objective of identifying physical and mental well-being in this vulnerable population. Syrian refugees were recruited through a Houston-based non-profit organization which facilitates re-settlement of Syrian refugees in the Houston area. Online survey was conducted using psychometrically valid and reliable instruments for measurement of mental well-being. Measures involved; Self-Report Questionnaire, Afghan Symptom Checklist, and Perceived Stress Scale, for evaluation of mental well-being, distress associated with war, violence, displacement and perceived stress, respectively. Arabic translations were utilized for all measures. Data were collected from a total of 74 Syrian refugees (35 males, 39 females). Participants were between 22-60 years old. Our data suggest that Syrian refugees are highly vulnerable for mental health problems and trauma-related distress symptoms, particularly, females who reported higher distress and stress symptoms when compared with males. Funding: This work was supported by the University of Houston Grants to Enhance Research on Racism (GEAR).

Longitudinal assessment of predator scent-induced disturbances in emotion and corticosterone in female mice.

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Predator scent exposure is a natural, traumatic stressor, frequently used in rodents to model symptoms of posttraumatic stress disorder (PTSD). While some aspects of this disease are relatively well investigated in animal models, dynamic changes in behavioral and neurobiological parameters in the aftermath of a traumatic experience have received insufficient attention. However, this is important considering that the majority of human trauma victims shows symptoms immediately after the trauma, which cease over time as the individual recovers. Only a vulnerable subgroup will continue to show symptoms and eventually develop chronic PTSD. We therefore presented female C57BL/6 mice with predator scent or water as a control and measured anxiety, body weight as well morning and evening corticosterone levels at three significant time points: before the trauma, immediately and two weeks after the trauma to investigate baseline levels, acute response and course of recovery or chronification. Further experiments in males are on the way to investigate sex differences in posttraumatic (mal)adaptation. Our preliminary results show increased levels of locomotor activity after the traumatic experience, which recover with time. Of note, we observed a large inter-individual variability in anxiety two-week after predator scent exposure. Of interest, these anxiety levels were associated with the changes in locomotor activity immediately after the traumatic experience. The high anxiety subgroup (formed by median split) showed a less pronounced increase in locomotor activity than the low anxiety subgroup. We believe that these subgroups represent vulnerable and resilient mice and will test this hypothesis in future experiments. Our present and future findings might be helpful for the early identification of individuals at risk of a pathological development in the aftermath of a trauma. Since this is essential for patient-tailored interventions, our longitudinal experimental design thus bears significant translational value. Funding: LSA-fellowship to IM.

Gonadal type and sex chromosome complement contributions to aversion-resistant alcohol intake.

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Alcohol use disorder (AUD) is a substantial health problem that affects both men and women in the USA. AUD is characterized by binge and compulsive-like drinking where individuals will continue to drink despite negative consequences. Higher rates of women are reporting that they are participating in more high-risk drinking behaviors. To model compulsive-like alcohol intake, preclinical aversion-resistant paradigms such as the drinking in the dark (DID) task have been developed where quinine, a bitter tastant, is added to an alcohol solution. We have shown using this task that female mice will consume more 15% ethanol (EtOH) compared to males during escalation. Despite observing behavioral differences, the mechanisms associated with greater drinking in females is unknown. Here, we used the Four Core Genotypes (FCG) mouse model to investigate the contribution of gonadal type (ovaries vs. testes) and sex chromosome complement (XX vs XY) to aversion-resistant intake. 26 FCG mice with ovaries (Sry-) had access to water or 15% EtOH in a two-bottle, limited access DID paradigm for up to 4 h and for a total of 15 drinking sessions. Aversion-resistance was tested by adding quinine to the ethanol bottle. Quinine concentrations escalated from 100, 250, to 500 ÂµM for 5 drinking sessions per concentration. The estrous cycle was monitored on 4 out of the 5 drinking sessions. Bottles were weighed every 30 min, 2 h, and 4 h into each drinking session. Bottles were alternated daily. No differences in alcohol consumption were observed between groups during escalation. Mice with the XY chromosome complement preferred alcohol more during escalation. Further, mice with the XY chromosome complement consumed more of 250 and 500 uM quinine exhibiting greater aversion-resistance than mice with the XX chromosome complement (who only exhibited aversion-resistance at 100 uM quinine). These findings indicate that compulsive-like alcohol intake may be differentially regulated by sex chromosomes and concur with the literature which shows that XY chromosomes are associated with habitual responding for EtOH. This study helps lay the foundation for novel future avenues of investigation of the mechanisms that contribute to female vulnerability to alcohol.

Neurocircuitry of social fear extinction. Involvement of septal oxytocin signaling?

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Social anxiety disorder (SAD), is the second most common anxiety disorder and characterized by fear and avoidance of social situations. Current treatments include cognitive-behavioral therapy, combined with rather unspecific pharmacotherapy. Thus, understanding the neuronal mechanisms underlying SAD is of utmost importance to develop new therapeutic strategies. Using the social fear conditioning (SFC) paradigm, we identified oxytocin (OXT) signaling within the lateral septum (LS) as a critical regulator of social fear extinction. The LS is a subcortical brain region that is reciprocally connected with various brain regions like the amygdala and the hypothalamus, which control different drive states and motivated responses. Therefore, the LS is thought to serve as an essential converging point, that relays incoming cognitive information to downstream affective regions that then adjust the behavioral output in response to varied environmental stimuli. In the central nervous system, OXT receptors (OXTR) can be found in high levels in neurons of the LS. OXT-signaling within the LS reverses social fear, but the exact signaling mechanism and downstream targets of OXTR-positive neurons in the LS in the context of social fear extinction is still unknown. We were able to reduce social fear expression in male and female CD1 mice, by infusing carbetocin, a biased OXTR-Gq agonist, bilaterally into the LS. This suggests, that Gq-signaling might be responsible for actions of OXT within the LS. Additionally, we will use viral tracing and immunohistochemistry in combination with an OXTR-Cre mouse line to not only precisely map the up- and downstream targets of the OXTR-expressing neurons within the LS, but also characterize their biochemical nature. Following this descriptive approach, we then aim to functionally modulate these neurons and their downstream targets using pharmacological, chemogenetic and optogenetic approaches within the framework of the SFC paradigm and test their effects on fear expression during social fear extinction. This study may provide a deeper understanding on how septal oxytocin signaling modulates emotional states, and might shed light on the mechanisms that underlie the extinction of social fear. Supported by Deutsche Forschungsgemeinschaft (Ne465-33-1; GRK 2174)

Sexual bias in the altered expression of myelination factors in mice with partial genetic deficiency of tryptophan hydroxylase 2 and pro-aggressive effects of predation stress

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A decrease in the CNS serotonin (5-HT) arising from genetic and environmental factors can contribute to behavioral abnormalities and the risk of neurodevelopmental disorders in humans. Male mice with partial deletion of the key enzyme in brain 5-HT synthesis tryptophan hydroxylase-2 (Tph2+/-) subjected to stress exhibited excessive aggressiveness, impulsivity, and hyperactivity. A similar phenotype is observed in Tph2-/- male mice. Aggressive and impulsive behaviors may arise from myelination deficits, evident in some neurodevelopmental disorders. Similar deficits in frontostriatal tracts were also associated with such behaviors in animals. Developmental deficit of 5-HT may cause abnormalities in myelination, which are often accompanied by neuroinflammation, and it also may contribute to altered behavior. Here, male, and female Tph2+/- mice were subjected to rat exposure and studied for their emotionality and social/aggression-like behaviors. Myelination proteins, myelin basic protein (Mbp), and proteolipid protein-1 (Plp1), and a marker of neuroinflammation interleukin-1 beta (IL-1 beta) were studied in the brain. Stressed Tph2+/- mice of both sexes exhibited elevated aggressive and dominant behavior and lowered sociability was observed in stressed male Tph2+/- mice. We found decreased expression of Mbp and Plp1 in the hippocampus of non-stressed Tph2+/- mice. Mbp expression in females'; prefrontal cortex was increased in non-stressed mutants in comparison to non-stressed wild-type mice, and stress lowered both Mbp and Plp1 expression in Tph2+/- females. No significant stress-related changes were observed in male mice. In both sexes, the expression of IL-1 beta was not changed. Thus, an interaction of neuronal serotonin deficiency and environmental adversity in Tph2+/- mice results in behavioral changes and sex-specific alterations in the expression of myelin proteins, which may underlie some of the traits, which are found in this model.

The Interaction of COMT Val158Met and Education Shapes Prefrontal Functions: The Case of Working Memory Updating.

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The role of dopamine in working memory (WM) has been demonstrated by many previous studies. However, it is unclear how COMT Val15Met, which is a marker for prefrontal dopamine capacity, contributes to WM updating when academic environment is considered. COMT Val158Met Met-allele which results in lower COMT activity, higher levels of dopamine, can enhance WM performance. In contrast, Val-allele has been associated with better cognitive plasticity, which can perhaps lead to higher susceptibility to academic environmental influences. In a recent study (Tamm et al., 2021), education significantly predicted WM updating, so that with each additional level of education, correct response times increased. However, that study did not explore the interactions of COMT Val148Met and education. Thus, in the present follow-up, the interaction of COMT Val158Met, and education in WM updating efficiency was explored to understand the role of gene-environment interactions in shaping the prefrontal functions. WM updating efficiency was operationalized as response times for correct responses (speed and accuracy trade-off). A population representative sample of young adults (n=498) completed a 2-back WM task with facial expressions. Correlation analysis and mixed modelling approaches were used. The correlations between educational attainment level and WM efficiency (speed of correct responses) showed that education did not significantly correlate with WM efficiency in Met/Met genotype (p=0.132). The positive correlation between education and updating response times was small but statistically significant in Val/Met genotype (Pearson's r(138)=0.180, p=0.004), and moderate in Val/Val genotype (Pearson's r(138)=0.225, p=0.02). The role of sex was further explored in linear mixed models. These results demonstrate that subjects with COMT Val-allele seem to be more sensitive to the effects of education. While higher education is associated with slower WM updating in subjects with Val-allele, there is no effect of education on WM updating in Met/Met genotype. These results contribute to understanding the variability in COMT Val158Met effects on cognitive performance. The interaction of COMT Val158Met and education suggests that academic environment can shape the neural substrate for WM.

Ameliorating the effects of an environmental toxin in a Drosophila model of Parkinson's Disease

Dionne Williams, Hakeem Lawal

Parkinson's Disease (PD) is a neurodegenerative disorder characterized in part by the selective loss of dopaminergic neurons in the substantia nigra pars compacta. Although the precise cause of PD is not yet fully understood, environmental factors are known to contribute to the etiology of a vast number of cases. Rotenone, a pesticide that inhibits Complex 1 of the Mitochondrial Electron Transport Chain, is one such toxin. Importantly, there is no known cure for PD and effective treatment options are severely limited both in number and efficacy. We are interested in developing neuroprotective strategies that may lead to more effective treatments for the disease. This project studies the effects of rotenone-induced toxicity in adult Drosophila melanogaster and the neuroprotective capacity of dacarbazine, a possible anti-PD drug that was identified in a previous pharmacological screen. We hypothesized that dacarbazine will confer both organismal and neuroprotection against rotenone-induced toxicity and mitochondrial dysfunction. And we report that treatment with dacarbazine led to a partial rescue of organismal lethality induced by rotenone. Further, we measured the effect of rotenone on mitochondrial oxygen consumption rate (OCR) using the Seahorse Analyzer and tested whether treatment with dacarbazine can ameliorate the effects of the rotenone inhibition of the mitochondria and we present preliminary data on the effect of dacarbazine on OCR in fly heads. In sum, our report shows the utility of a potential neuroprotective chemical against a model of PD and suggests a possible neuroprotective mechanism against the diseases. Funding Acknowledgements: HGBI Title III Grant, NSF MRI Grant, and Delaware Economic Development Office. Grant #106 to H. Lawal (PI).

Exercise protects against synergism of lipopolysaccharide and chronic restraint stress in female mice

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Lippopolysaccride (LPS) is a neuroinflammatory model for Parkinson's disease (PD), where dopamine neurons (DA) in the substantia nigra (SN) die. LPS and chronic restraint stress (CRS) act on similar mechanisms to induce reactive microglia (MG), which may synergistically increase their toxic effects. Exercise, a potent behavioral therapeutic intervention, may increase resiliency via trophic factors. This is an important novel study, as there are sex differences in reactivity of MG, yet no research has examined chronic and/or synergistic effects of LPS and CRS in females. C57/Bl6J female mice were pair housed in sedentary standard housing (SED), or with exercise wheels (EX), and assigned to: no stress (NS), LPS, LPS+CRS, CRS. LPS groups received 1 mg/kg LPS i.p., once a wk for 3 wks. CRS groups received randomized restraint stress for 2 hrs daily for 3 wks. At 1- and 3 mths later, Brain derived neurotropic factor (BDNF) was assessed in CA 1-3 in stress vulnerable dorsal (dHp), versus resistant ventral hippocampus (vHp). No differences in BDNF in v- versus d- Hp were seen at either time point. In SED groups there was signif. lower BDNF at 3 mths in all LPS groups across CA1 & 2, d and vHP p=0.01. In the CA3 dHp SED LPS+CRS had signif. lower BDNF at 3 mths p=0.03. BDNF was lower at 3 mths in the vHp CA3 region for LPS groups p<0.02. In EX groups there was no signif. difference between time points or between regions, suggesting EX increases resiliency. To assess vulnerability to PD, DA neurons, and pro-inflamm MG in the SNpc were quantified. In SED groups there was no signif. difference of IBA1/COX2 between timepoints, yet in EX LPS there were increased levels at 3 mths p=0.001. Interestingly within SED there were higher levels in LPS+CRS than LPS at 3 mths p=0.002, while there were no differences for EX groups, suggesting EX protects against delayed toxic effects. There was no signif. difference in Tyrosine hydroxylase across timepoints, treatment, and activity level, indicating the toxic effects of LPS may occur later. These data show that LPS and CRS synergistically increase pro-inflamm MG at 3 mths, and that EX protects against decreased levels of BDNF in stressed groups across time points, reducing the toxic effects of multiple stressors. Funded by Schapiro Undergraduate Research Fellowship Award, RMC

Individual differences in fear extinction: role of the cholinergic system.

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Individuals with Posttraumatic Stress Disorder suffer from dysregulated fear extinction. Rodents similarly display individual differences in fear extinction whereby extinction competent (EC) rats extinguish a fear memory and extinction resistant (ER) rats show enduring fear responses despite repeated exposure to a conditioned stimulus in a safe environment. Glutamatergic and cholinergic inputs converge in the basolateral amygdala (BLa), but how the cholinergic system regulates glutamate to affect fear extinction is unknown. In naÃ¨ve rodents we showed that the suppression of corticoamygdalar inputs to the BLa induced by muscarinic acetylcholine receptor (mAChR) activation varied between individuals, so we hypothesized EC/ER rats would differ in mAChR corticoamygdalar inhibition. To test this, male Long Evans rats were divided into EC/ER groups based on freezing during extinction learning. Ex-vivo slice electrophysiology was subsequently used to record cortically evoked BLa responses with increasing doses of mAChR agonist, muscarine. Muscarine dose-dependently suppressed corticoamygdalar transmission and this effect was reversed by the muscarinic antagonist atropine. At the low dose, ER rats displayed greater muscarinic inhibition of glutamatergic input than EC rats, suggesting divergent regulation by mAChRs in EC/ER rats. To examine individual differences in inhibition by endogenous acetylcholine, we characterized fear behaviors and brain cholinergic markers in a transgenic ChAT::Cre rat model that underwent Pavlovian fear learning and extinction. We found group differences in freezing during context recall and cued extinction learning, ultrasonic vocalizations, and cholinergic markers in the BLa and basal forebrain of Cre+ rats compared to Cre- transgenic rats. These differences between Cre+ and Cre- rats suggest transgenic ChAT::Cre rodent lines, while valuable tools to study the cholinergic system, are not optimal for examining cholinergic regulation of individual differences in fear behaviors. Support: VA Merit Award I01BX001374 (MAW), ASPIRE1 Award (SCT), and Magellan Scholars/Capstone Awards (GG)

Amygdalostriatal transition zone neurons encode sustained valence to direct conditioned behaviors.

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The ability to respond appropriately to stimuli that predict rewards or punishments lies at the core of evolutionary fitness, and is disrupted in a number of neuropsychiatric disease states. Although relatively unexplored, the amygdalostriatal transition zone (ASt) may play a crucial role in parallel with the amygdala to mediate associative learning and behavioral responses to salient stimuli. Like the amygdala, the ASt receives converging input from two major streams of sensory information, the thalamic and cortical pathways. However, the downstream projections of the ASt are distinct from the canonical outputs of the amygdala complex, and are integrated with striatal circuits involved in action selection. Despite this intriguing circuit connectivity, the function of the ASt is almost completely unknown, resulting in a major gap in our knowledge of circuits underlying motivated behaviors. In the present study, that the ASt constitutes a unique, genetically distinct region from adjacent striatal brain regions, and has a greater proportion of Drd2+ neurons than the tail of striatum (TS). Using in vivo electrophysiology we show that ASt neuron responses to a shock-predicting cue are significantly greater following fear conditioning in 'paired group' mice compared with 'unpaired group' controls. We also identify distinct populations of ASt neurons that encode opposing conditioned responses to cues predicting rewarding and aversive stimuli. Preliminary calcium imaging data further suggests that D2+ ASt neurons show increased conditioned cue responses following fear conditioning. Finally, in loss-of-function experiments we find that optogenetic inhibition of a D2+ ASt neurons reduces conditioned responses to a shock-predicting cue. Consequently, we believe that the ASt may be an overlooked and critical structure of the amygdala complex that contributes to behavioral responses to conditioned stimuli.

Selective inhibition of PDE4B reduces methamphetamine-taking in two C57/BL6 mouse substrains.

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Methamphetamine (MA) is a highly addictive psychostimulant drug and the number of MA-related overdose deaths has reached epidemic proportions, particularly within the U.S. There exists no approved pharmacotherapy for treating MA use disorder; however, evidence supports the potential utility of targeting neuroimmune function using non-selective phosphodiesterase 4 (PDE4) inhibitors. Off-target emetic effects associated with non-selective PDE4 inhibitors have prompted the development of selective PDE4 isozyme inhibitors for treating neuropsychiatric conditions. Herein, we examined the efficacy of pretreatment with the selective PDE4B inhibitor A33 (0.0-1.0 mg/kg, IP) on the maintenance of oral MA self-administration in mice. As both sex and substrain differences in neuroimmune function are reported in mice, we compared the dose-response functions for A33 effects on MA-taking in C57BL/6J (B6J) and C57BL/6NJ (B6NJ) mice of both sexes. We first trained mice to nose-poke for 200 mg/L MA, then examined for sex and substrain differences in the dose-response function for oral MA intake (25-3200 mg/L; 3-5 days/concentration). This analysis identified 400 mg/L MA as a concentration that lies on the ascending limb of the dose-response function that engendered a moderately high and stable level of responding in all groups. Maintaining self-administration behavior on 400 mg/L MA, we employed a within-subjects design to examine the effect of A33 pretreatment (30 min) on MA-taking with the order of A33 dosing pseudo-randomly assigned across animals. A33 injections were administered every 3-5 days when MA intake had returned to pre-injection levels. During training and MA dose-response testing, B6J mice and female mice exhibited more MA reinforcement and intake than their B6NJ and male counterparts. Pretreatment with A33 dose-dependently reduced MA reinforcement and intake in mice of both sexes and substrains. These findings provide the first evidence that pretreatment with a selective PDE4B inhibitor effectively reduces well-established MA-taking behavior in both male and female mice of two genetically distinct substrains. If relevant to humans, these results posit the potential clinical utility of A33 or other selective PDE4B inhibitors for treating MA use disorder.

The effects of novel high-CBD cannabis on neuroanatomy in the Long-Evans rat.

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Cannabis, a drug derived from the plant cannabis sativa, has been used medicinally and recreationally for thousands of years. One particular phytocannabinoid in cannabis, cannabidiol (CBD), has many reported health benefits, including anti-inflammatory and analgesic properties, and is often sought after to serve as a complementary modality. It is critical however, that these translational applications be supported by a foundational understanding of the effects of this drug on the brain and on behaviour. This project uses a rodent model to examine the neuroanatomical as well as behavioural outcomes following exposure to novel cannabis strains with high-CBD and low-THC (tetrahydrocannabinol; the primary psychoactive component of cannabis). We hypothesize that there will be limited influence of these cannabis extracts on both morphological measures of brain tissue and subsequently, animal behaviour. To address our hypothesis, Long-Evans rats were orally administered a unique high-CBD, low-THC cannabis extract in peanut butter at one of two dosages, a low dose of 10 mg/kg, or a high dose of 40 mg/kg, for 10 days in adulthood. Control animals received no cannabis extract but followed an identical dosing/testing paradigm. Behavioural testing was conducted prior to dosing, in adolescence, and following dosing in adulthood, using a battery of well-established tests sensitive to changes in the prefrontal cortex and hippocampus, two brain areas susceptible to the effects of cannabis. Following behavioural testing, animals were euthanized and their brains extracted for anatomical analysis. Measures of prefrontal and hippocampal synaptic connectivity and dendritic morphology, as well as gross measures of cortical thickness and thalamic area were taken from Golgi-Cox stained brain tissue. These cannabis extracts appear to have little effect on neuroanatomy or animal behaviour (including measures of anxiety-like behaviour, locomotor activity, fine motor function and spatial learning and memory). Thus, with limited anatomical and behavioural consequence, these high-CBD cannabis extracts present themselves as viable options for medicinal application. This work was funded by the Natural Sciences and Engineering Research Council of Canada, and Branch Out Neurological Foundation.

Real-time pharmacokinetic and pharmacodynamic measurements of drugs within the brains of freely behaving rats.

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The drug cocaine evokes complex neurochemical responses that mediate their influence of neural circuits underlying behavioral control, such as the dopamine pathways that we now know drive reward anticipation and locomotor behavior. In contrast, the anesthetic drug procaine functions as both a local anesthetic in the periphery, and a general anesthetic when it reaches the brain, affecting breathing, heart rate, and locomotion. However, the time resolution with which existing methods can measure these drugs is orders of magnitude poorer than the resolution with which physiological responses to these drugs occur. In response to this problem, our group has recently developed a new technology, electrochemical aptamer-based sensors (E-AB sensors), which are the size of a human hair and can detect physiologically relevant concentrations of specific drugs in-situ, in awake, freely behaving animals. Here, we tested this new technology on rats that have been administered either cocaine or procaine intravenously and measured the concentrations of these drugs within the lateral ventricle concurrent with monitoring locomotor activity. We successfully measured the concentration of cocaine and procaine every 10 s for 2 hours and generated a full pharmacokinetic and pharmacodynamic profile for these drugs within the lateral ventricle. As cocaine concentrations increased (from 0 to 5 Î¼M), they directly corresponded with increased locomotor activity and stereotypy, whereas increased levels of procaine in the lateral ventricle coincided with decreased heart rate, oxygen saturation (SPO2), and locomotion. In conclusion, we have developed a novel, potentially revolutionary, technology that can measure the pharmacokinetics and pharmacodynamics of psychoactive drugs within the brains of awake, freely behaving animals. Funding was provided by the National Institutes of Health R01EB022015 and the W.M. Keck foundation.