1. **Glyoxalase 1 involvement in alcohol drinking and alcohol-motivated behaviors**

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The gene glyoxalase 1 (Glo1) has been previously implicated in anxiety- and depression-like behaviors in mice, and also in binge-like alcohol consumption. The substrate of the enzyme GLO1 is methylglyoxal (MG), which acts as a partial agonist at GABA-A receptors. Reducing GLO1 activity through genetic knockdown Glo1 or through direct inhibition of the enzyme leads to a buildup of MG and presumably increased GABAergic transmission, which accounts for the observed behavioral effects. Overexpression of Glo1 leads to an increase in binge-like alcohol consumption, whereas knockdown of Glo1 or treatment with a GLO1 inhibitor reduces consumption. However, treatment with a Glo1 inhibitor does not change the dose-response curve for alcohol locomotor stimulation/sedation, and genetic manipulation of Glo1 levels does not appear to affect locomotor response to alcohol either, suggesting that GLO1 manipulation does not simply enhance sensitivity to alcohol’s effects. In an intracranial self-stimulation (ICSS) paradigm, a 1.5 g/kg dose of alcohol lowered response thresholds (i.e. enhanced reward) whereas a GLO1 inhibitor administered alone had no effect on thresholds. This suggests that GLO1 inhibitors may have a low abuse liability, providing further support for this system as a target for the treatment of AUDs and comorbid disorders. In ongoing ICSS studies, we are examining whether GLO1 inhibition can block the reward enhancing effects of alcohol, and whether reward enhancement by alcohol is altered in Glo1 overexpressing or knockdown mice. These experiments further demonstrate the role of Glo1 in alcohol-mediated behaviors and begin to elucidate why Glo1 manipulation alters alcohol consumption.

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2. Effect of acute emotional stress on behavior, level of plasma corticosterone and brain monoaminergic systems in tumour necrosis factor-α knockout mice

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Tumour necrosis factor-α (TNF) is a pro-inflammatory cytokine that plays important role not only in immunity but also in the normal functioning of the central nervous system.

The aim was to evaluate the involvement of TNF in acute restraint stress effect on behavior, level of plasma corticosterone and brain monoaminergic systems. Experiments were carried out on adult males of C57BL/6 (WT) mice and mice deficient for TNF (TNF KO) generated on a genetically pure C57BL/6 background. Locomotor activity and anxiety-like behavior were evaluated in open-field test. The levels of noradrenaline (NA), dopamine (DA), serotonin (5-HT) and their metabolites were estimated in brain using HPLC. The levels of plasma corticosterone were measured by ELISA kit.

Acute stress led to reduction of horizontal (distance traveled) and vertical (number of rearings) activities only in TNF KO mice (p<0.001), while no influence of stress on time spent in center of open-field was found in both strains. Stress-induced rise of plasma corticosterone was more intensive in TNF KO than in WT mice (p<0.001). Restraint stress augmented the 5-HIAA/5-HT turnover rate in all investigated brain structures of both strains (p<0.01), while only TNF KO mice demonstrated increased DA level in the hypothalamus and striatum (p<0.05) and increased DOPAC/DA rate in the striatum and substantia nigra (p<0.05). The restriction reduced NA level in the hypothalamus of both strains (p<0.05) and in the hippocampus of TNF KO mice (p<0.01).

Consequently, TNF KO mice showed higher sensitivity to acute emotional stress effect compared to WT mice.
3.

Toward Understanding Tetracycline Analog Efficacy of Alcohol Use Disorder Treatment

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Current treatment of Alcohol Use Disorder (AUD) is inadequate in addressing shared international goals of providing effective therapeutic management or a cure. Such a dearth of effective intervention for AUD results in a global health challenge with upwards of 15% of health issues attributed to alcohol consumption in some countries. Recently, we showed preclinical evidence that some tetracycline analogs were effective in reducing alcohol consumption, withdrawal symptoms and associated pain responses. Believed to act, at least in part, on the innate immune system, the specific mechanisms of action are not well-understood, including for known sex differences in efficacy. Here, we used an amalgamation of anatomical, behavioral, genetic, molecular and pharmacological tests in mice to suggest that minocycline and tigecycline 1) act directly in the CNS and not through reduction of peripheral cytokine signaling; 2) are not affected by knock-out of Cx3Cr1; and 3) may interact with matrix metalloproteinase function resulting in sex differences in efficacy.

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Genetic and environmental contributions to individual differences in emotion regulation: Preliminary results from the Romanian Twin Registry

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The habitual use of emotion regulation strategies has multiple affective, cognitive and social implications. However, the genetic and environmental factors that contribute to individual differences in emotion regulation have not been examined until now. The present twin study was designed to investigate the heritability of habitual reappraisal and suppression, based on pilot data from the recently initiated Romanian Twin Registry. The sample included 25 monozygotic twin pairs and 26 dizygotic twin pairs, for which zyosity was established based on self-report. Individual differences in emotion regulation were assessed using Gross and John’s Emotion Regulation Questionnaire. Using structural equations, six biometric models with different combinations of additive (A) and non-additive (D) genetic effects, and shared (C) and specific (E) environmental effects were fitted to the twin data. The best fitting model for habitual reappraisal was the AE model, with an estimated additive genetic variance of .40 and specific environmental variance of .53. The best fitting model for habitual suppression was the CE model, with an estimated shared environmental variance of .51. Specific environment also contributed to habitual suppression, but its magnitude could not be reliably estimated due to the small sample. Therefore, these preliminary results suggest a contrast between habitual reappraisal and suppression, with the former involving moderate heritability and specific environment, and the latter only environmental factors.
5.

Long-lasting consequences of early life stress in mice: changes in gene expression and H3K4me3 profile.


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Stressful events in early postnatal period have critical consequences for the individuals’ life and can increase later risk for the development of psychiatric disorders. We have investigated the influence of early life stress on expression of genes of glutamatergic and glucocorticoid systems in prefrontal cortex and hypothalamus in adult male mice. We explored the effects of two types of stress: separation of pups from their mothers for 3 hours per day (maternal separation, MS) and for 15 min daily (handling, HD) during the first 2 weeks of life when compared with nonhandled control. We have found decreased expression of \textit{Grin2b} gene in MS group and increased expression of \textit{Avp} gene in both MS and HD groups in hypothalamus. In prefrontal cortex increased expression of \textit{Fkbp5} has been revealed just as in MS group so in HD group. Activation of glucocorticoid system in both MS and HD conditions indicate stress protection response to aversive life events. To define probable epigenetic changes mediating long-lasting effects of early postnatal stress we analyze H3K4me3 ChIP-seq profiles in prefrontal cortex. We have revealed significant differences in H3K4me3 levels only between MS and control groups in 10 chromatin loci. The revealed loci are located in the promoters of 16 genes, two of them (\textit{Pip4k2a}, \textit{Ddias}) showed significantly increased expression in MS group compared to control. H3K4me3 levels have not changed in promoters of the studied genes of glutamatergic and glucocorticoid systems. Our results indicate that early postnatal stress have long-lasting consequences on genes expression and epigenetic status in adult mice.

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Systems genetic analysis and fine mapping in a reduced complexity cross rapidly leads to the identification of compelling candidate genes underlying behavioral addiction traits

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ABSTRACT

Opioid addiction is a heritable substance use disorder with an unknown genetic etiology. Mammalian model organisms permit a comprehensive approach to bridging genetic variation with neurobiological mechanisms of addiction-relevant behaviors. The closely related C57BL/6J
and C57BL/6NJ substrains show extremely limited genetic diversity, yet show differences in alcohol consumption, binge eating, psychostimulant-induced locomotor activity, and naloxone aversion. Here, we replicated strain differences in psychostimulant-induced locomotor activity with methamphetamine and used quantitative trait locus (QTL) mapping to identify a QTL on chromosome 5 (52-99 Mb) that co-mapped with a striatal cis-expression QTL (eQTL) for Gabra2 (71 Mb). We also co-mapped QTLs for opioid-induced locomotor activity and withdrawal induced by the mu opioid receptor agonist oxycodone to distal chromosome 1 (LOD=4.7-9.8; 152-181 Mb). We backcrossed F₂ mice based on this QTL genotype to C57BL/6J to fine map a region containing a well-known neurobehavioral QTL “hotspot” (chr1: 172-178 Mb). Shared haplotype analysis of C57BL/6NJ and classical inbred strains combined with cis-eQTL analysis of F₂ mice identified two strong positional candidate genes for oxycodone behaviors - Aim2, and Rgs7. Pathway analysis in opioid withdrawn F₂ mice identified two major hub genes underlying opioid dependence - Mapt and App. Finally, we identified a major QTL for naloxone-induced aversive behavior on chromosome 18 (LOD=6.2; 35-62 Mb) near a striatal cis-eQTL for Onecut2 (64 Mb), a gene involved in neurodevelopment of the locus coeruleus. To summarize, systems genetics and fine mapping in a reduced complexity cross revealed compelling candidate genes underlying addiction traits.

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7.

**Pain catastrophizing, neuroticism, fear of pain, and anxiety: defining the genetic and environmental factors**

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The objective of the present study was to establish the heritability of pain catastrophizing and its subdomains of helplessness, magnification, and rumination. To further explore the genetic and environmental sources that may contribute to pain catastrophizing as well as to its commonly reported psycho-affective correlates, including neuroticism, anxiety sensitivity, and fear of pain. N = 2733 twin individuals from the TwinsUK registry were subject to univariate and multivariate twin analyses. Validated questionnaires including the Pain Catastrophizing Scale, the Pain Anxiety Symptom Scale, the Ten Item Personality Index, and the Anxiety Sensitivity Index were used to assess the study variables. Moderate estimates of heritability for pain catastrophizing (35%) and the three subdomains of helplessness (35%), rumination (26%), and magnification (35%) were
detected. The high correlations observed between the three subdomains were explained mainly by overlapping genetic factors, with a single factor loading on all three phenotypes. High genetic correlations between pain catastrophizing and its psycho-affective correlates of fear of pain and anxiety sensitivity were found, while the genetic overlap between neuroticism and pain catastrophizing was low. Each measure of negative affect demonstrated relatively distinct environmental contributing factors, with very little overlap. This is the first study to show shared genetic factors in the observed association between pain catastrophizing and other measures of negative affect. Our findings provide deeper insight into the aetiology of pain catastrophizing and confirm that it is at least partially distinct from other measures of negative affect and personality that may influence the development and treatment of chronic pain conditions.

8.

5-HT1A and 2 Adrenergic Receptors Modulate Anxiety-like Behavior and Impulsivity in Selectively Outbred Long-Evans rats

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Trait anxiety and drug addiction often overlap in clinical populations as evidenced by high comorbidity rates between anxiety and substance use disorders. Impulsive behaviors also map on to escalation from casual to uncontrollable drug use, which is characteristic of addiction. The present study aims to elucidate whether Long Evans trait anxiety male rats that are hypersensitive to amphetamine (AMPH) also show varying impulsivity levels in an operant paradigm. Animals were outbred and trait anxiety was phenotyped utilizing the elevated plus maze (EPM); sensitivity to AMPH was evaluated at a low dosage of 0.5mg/kg, i.p. To measure impulsivity, 6th generation male Long Evans rats (N=16) were exposed to a differential reinforcement of low rate of responding (DLR: 15sec) operant schedule. Additionally, protein receptors implicated in anxiety and drug addiction neurocircuitry were measured. HAn rats exhibited higher levels of anxiety-like behavior, showing more time on open arms of the EPM. HAn males also showed increased locomotion after exposure to AMPH relative to low-anxiety rats. HAn rats displayed increased impulsivity levels on behavioral measures of DLR including: total rewards attained, inter-response times without reward present, and burst ratios. Cellular analyses revealed in HAn rats an increase in 5-HT1A protein levels in the medial prefrontal cortex and nucleus accumbens, along with an increase
in -2 adrenergic receptor proteins in the locus coeruleus. These results suggest that high-anxiety exhibiting rats are hyper-sensitive to AMPH and also display impulsivity levels that are associated with changes in corticolimbic protein levels such as 5-HT1A and -2 adrenergic receptors.

9.

An altered neurodevelopmental profile in mice deficient for autism-associated Neurexin1 gene: communicative and motor aspects at an early stage
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Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disorders with early onset of symptoms, characterized by socio-communicative deficits and patterns of stereotyped behaviours. Although the causes of ASD remain unclear, evidence strongly supports the role of genetic factors in their aetiology, including mutations in Neurexin (NRXN) family genes. Our study aimed the behavioural phenotyping of mice with deletion of Nrxn1α gene encoding for a neuronal presynaptic cell-adhesion molecule in order to identify autistic-like features, as soon as possible during the early developmental period. We evaluated the ontogenic profile of the vocal response during the first weeks of life through a detailed analysis of the ultrasonic vocalizations. Ultrasonic vocalizations are emitted by mouse pups in response to isolation from the mother and littermates and considered a suitable tool for the identification of early communication deficits in autism mouse models. Moreover, since motor dysfunctions can predict the onset of the other symptoms in ASD, we performed a fine-grain characterization of spontaneous motor behaviours, recorded simultaneously with the ultrasonic vocalizations. This is the first vocal and motor evaluation of Nrxn1α mutant pups that allows identification of autistic-like phenotypes at an early developmental
stage. Our results indicate that an altered profile is detectable in the emission of ultrasonic vocalizations and acquisition of specific motor patterns in $Nrxn1\alpha$ mutant pups, in line with behavioural phenotypes of ASD children. Our findings suggest that the $Nrxn1\alpha$ gene has an important neurodevelopmental function and its deletion causes specific early behavioural abnormalities.
ADOLESCENT SOCIAL STRESS DIFFERENTIALLY IMPACTS AFFECTIVE BEHAVIORS AND NICOTINE SENSITIVITY IN C57BL/6J AND BALB/cJ MICE

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Affective disorders and nicotine use are significant contributors to global morbidity and mortality as independent and comorbid diseases. Early-life stress, potentially via stress-induced hypothalamic-pituitary-adrenal axis dysregulation, can exacerbate both. However, little is known about the factors shaping susceptibility to comorbidity of these disorders. We examined the relationship between stress-induced changes to affect-related behaviors and nicotine sensitivity. Male and female mice (BALB/cJ & C57BL/6J) were exposed to either chronic variable social stress (CVSS) or control conditions during adolescence (PND 25-59). In adulthood, anxiety/depression-related behaviors were measured using the elevated plus-maze (EPM) and social interaction test (SI). Nicotine sensitivity was assessed via acute effects on body temperature, corticosterone production, locomotor activity, and voluntary oral nicotine consumption. We then characterized spontaneous GABA/glutamate transmission in the prefrontal cortex (PFC), basolateral amygdala, and nucleus accumbens (NAc), because these stress-sensitive regions regulate affect- and drug-related behaviors. Relative to controls, CVSS males exhibited reduced EPM open arm activity and increased social avoidance (male C57BL/6J). Alternatively, CVSS increased sensitivity to nicotine-induced locomotion during late-adolescence and adult voluntary nicotine consumption in BALB/cJ males, relative to controls. CVSS increased adult nicotine-induced corticosterone production in BALB/cJ males and reduced corticosterone production following injection stress in C57BL/6J males, relative to controls. Finally, CVSS decreased glutamate (PFC & NAc) and increased GABA (PFC) transmission in C57BL/6J males. Females were unaffected by CVSS. Results indicate that CVSS differentially impacts affect-related behavior/physiology or nicotine sensitivity in C57BL/6J or BALB/cJ males, respectively. Thus, changes in affect-related behavior/physiology and nicotine sensitivity following adolescent social stress are sex- and genotype-dependent.

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The Contribution of a Polygenic Risk Score and Experiences of Childhood Maltreatment to the Use of Maladaptive Coping Strategies

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Maladaptive strategies (e.g., alcohol use, aggression) used to cope with daily stressors can lead to negative consequences such as alcoholism, depression, interpersonal violence, or suicidal behavior. Engagement in maladaptive coping strategies is partly the result of individual differences in stress reactivity. Furthermore, the use of maladaptive coping strategies contributes to both frequent and prolonged HPA axis activation. Therefore, it is crucial to understand the factors that contribute to an individual’s use of maladaptive coping strategies to prevent or reduce negative consequences associated with aggression and alcohol use. Undergraduate students (N = 765; 56.3% female; 80.1% Caucasian; age M = 19.53 [SD = 2.26]) completed self-report questionnaires regarding demographics, alcohol use, aggression, perceived stress, and childhood maltreatment, and donated saliva for genotyping purposes. Genetic risk (i.e., association with lower transcriptional efficiency of serotonergic genes) was measured with a polygenic risk score created from five polymorphisms (HTR1B rs13212041, TPH2 rs4570625, SLC6A4 5-HTTLPR+rs25531, and MAOA uVNTR). Among females with higher reported exposure to childhood maltreatment (75th percentile), higher polygenic risk scores were indirectly associated with higher aggressive behavior via higher perceived stress [CI: 0.08, 0.69]. No significant effects were present for males or individuals with lower reported exposure to childhood maltreatment. Genetic risk was also not associated with alcohol use. These findings contribute to our understanding of the etiology of aggressive behavior resulting from increased stress and suggest that females with lower transcriptional efficiency of serotonergic genes are at greater susceptibility to negative environmental factors (i.e., childhood maltreatment).

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Genetically correlated reward and aversion traits across generations of selection for methamphetamine consumption

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Selectively bred lines for addiction-related traits provide a model for understanding genetic factors that contribute to susceptibility to drug use. We produced mouse lines that voluntarily consume high (MAHDR) or low (MALDR) amounts of methamphetamine (MA). Emergence of 2 genetically-correlated traits, MA-conditioned place preference (CPP) and taste aversion (CTA), across 3 generations of selective breeding was examined. Preference for the MA-paired floor was greater in MAHDR than MALDR mice, regardless of MA dose (0.5 or 2 mg/kg MA; F[1,339]=16.9, p<0.001, main effect of mouse line). Generation did not impact CPP for the MA-free preference test, but when mice were treated with MA prior to preference testing, the difference between the lines increased across generations (F[2,339]=3.461, p<0.05), due to increasing place aversion in later generation MALDR mice. Consumption of 0.2M NaCl after paring with MA injections (0, 2, 8 or 16 mg/kg for MAHDR; 0 or 2 mg/kg for MALDR) was used to measure CTA. Data across trials was used to characterize the lines and consumption on the final trial was used to characterize CTA across generations. MAHDR mice were resistant to MA-induced CTA, exhibiting a response only to 16 mg/kg (F[12,152]=2.1, p<0.05). MALDR mice showed robust CTA at 2 mg/kg (F[1,80]=33.2, p<0.001). Neither MAHDR nor MALDR mice exhibited changes in CTA strength across generations. Taken together, these data suggest that the genetic factors influencing MA-induced CTA are recruited early in selection, whereas additional factors may be recruited across generation that impact MA-induced CPP in the presence of MA treatment.

1Oregon Health & Science University, Portland, OR, 2Veterans Affairs Health Care System, USA. Funding Support: Department of Veterans Affairs I01BX002106, NIH NIDA P50DA018165
13.

**A comparative phenotypic analysis of paclitaxel-induced neuropathy in C57BL/6J and C57BL/6N mouse strains**

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Mouse substrains are a powerful resource for rapid discovery of genes and pathways regulating complex behaviors. In this study, we report that C57BL/6J (B6J) and C57BL/6NCrl (B6N) substrains, differ significantly in paclitaxel-induced peripheral neuropathy (CIPN). Paclitaxel is a drug commonly used for the treatment of breast, lung, and ovarian cancer. CIPN is one of the most common and serious adverse effects experienced by cancer patients treated with paclitaxel. We used a low-dose regimen of paclitaxel (2 mg/kg, every other day for a total of 4 injections or one cycle) in B6J (Jackson Lab) and B6NCrl (Charles River) male and female mice. Paclitaxel injection in B6J resulted in long-term mechanical and cold allodynia, which represents an important clinical manifestation of peripheral neuropathy in a majority of patients. However, no significant allodynia was detected after paclitaxel injection in the B6N substrain. Importantly, cisplatin, another anticancer agent that causes CIPN, also induced long-term mechanical allodynia in B6J but not B6N mice. Furthermore, no significant allodynia was detected in these two substrains after chronic constriction injury (CCI) of the sciatic nerve. Because changes in the density of peripheral nerve fibers (IENF) represent a hallmark of CIPN, we examined changes in peripheral nerve fiber density following paclitaxel treatment using immunohistochemistry (labeling with protein gene product 9.5; PGP9.5). At 28 days post-paclitaxel injection, B6J but not B6N mice treated with paclitaxel demonstrated a significant reduction in the density of IENFs compared to vehicle-treated mice. Together, these results suggest that B6 substrains will be useful for future genetic studies on chemotherapy-induced neuropathy.

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Transcription profile of animal model of seizures Wistar Audiogenic Rat
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Wistar Audiogenic Rat (WAR) is an animal model of epilepsy whose seizures are developed by acoustic stimulation. When submitted to a high intensity sound, the WAR animals exhibit generalized tonic-clonic seizures due to the activation of quadrigeminal plate. The seizure’s predisposition might be related to allele’s fixation generated by the strain selection process. The aim of this study was the identify genes related to these animals’ predisposition to seizures. The RNA-Seq from quadrigeminal plate was made from WAR and Wistar animals submitted to acoustic stimulation. The differentially regulated genes (DGR) were identified by EdgeR software and the results validation was performed by qPCR using both animals (WAR and Wistar divided in stimulated and naive, stimulus’ free animals). Considering the value FDR≤0,05 we identified 64 DGR in stimulated WAR. Among the genes evaluated by qPCR 4 genes stood out due to their transcriptional profiles, Acsm3 (F3,27=176,0), Gpr126 (F3,28=141,11), Rtel1 (F3,28=60,85) e Qdpr (F3,27=11,80). The Grp126 e Rtel1 genes were downregulated in WAR naive and stimulated when compared to the control groups. The Acsm3 were upregulated in WAR. The Qdpr gene was upregulated only in WAR animals that presented seizures. The data the from transcripts of Gpr126 and Qdpr genes were confirmed by western blot. Our results confirm that ictal events causes gene dysregulation on the quadrigeminal plate of WAR, and there is a basal differential regulation in lineage independent of the seizures. Reinforcing the hypothesis that the modulation of the epileptic phenotype is related to fixation of alleles by inbreeding.
A QTL on chromosome 1 modulates intermale aggression in mice

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Intermale aggression is a complex social behavior that is likely regulated by multiple genes. In this study the BXD recombinant inbred mouse strains (RIS) were used to map quantitative trait loci (QTLs) underlying behaviors associated with intermale aggression. Four hundred and fifty-seven males from 55 strains (including the parentals) were observed at an age of 13 ± 1 week in a resident-intruder test following 10 days of isolation. Attack latency was measured directly within a 10 minute time period and the test was repeated 24 hours later. The variables analyzed were the percentage of attacking males in a given strain (on days 1 and 2, and both days combined), as well as the attack latency on days 1 and 2. On day 1, 29% of the mice attacked, which increased
to 37% on day 2. Strain differences were highly significant for all variables measured, indicating significant heritability. From these data, we identified a significant QTL on chromosome 1 for attack variables and suggestive QTLs on mouse chromosomes 7, 11, 12, and 13 for both attack and latency variables. The chromosome 1 QTL interval maps to a gene sparse region. The most likely candidate gene modulating this trait is Htr2b which encodes the serotonin 2B receptor and has been implicated in aggressive and impulsive behavior in both mice and humans. mRNA expression data and phenotype correlation analyses show significant relationships with the amygdala and striatum, and with fear and anxiety.

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16.
THERMOREGULATION AND POSTSYNAPTIC 5-HT1A RECEPTORS IN MICE.

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5-HT receptors are generally involved in the control of body temperature. Activating 5-HT1A receptors by 8-hydroxy-2-(dipropylamino)tetrailin (8-OH-DPAT) leads to reduced body temperature. Unlike rats and humans, it is still a matter of debate whether the 5-HT1A receptor-mediated regulation also takes place on postsynaptic level in mice. In our group, within phenotyping a transgenic mouse line permanently overexpressing the 5-HT1A receptor in serotonergic projection areas (OE mice), Bert et al. (2008, PMID: 18396339) revealed exaggerated 8-OH-DPAT- provoked hypothermic response. Thus, the present study aimed at assigning clear thermoregulatory function to the postsynaptic 5-HT1A receptor in mice.
We used radio telemetry technique to monitor basal body temperature and hypothermic effects of 8-OH-DPAT (0.1 mg/kg – 4 mg/kg i.p.) in male OE mice in comparison to NMRI wild-type (WT) males. Additionally, we investigated whether reduction of serotonergic activity by pretreatment with the 5-HT synthesis inhibitor parachlorophenylalanine (PCPA; 100 mg/kg, i. p. on four consecutive days) would alter the effects of 8-OH-DPAT on body temperature. OE mice revealed lower levels of basal body temperature (36.15 °C) than wild types (37.18 °C). In both genotypes, systemic administration of 8-OH-DPAT dose-dependently decreased body temperature, being significantly more pronounced in mutant mice (-2.55 °C compared to -1.4 °C in WT mice). Dose-response curves of 8-OH-DPAT revealed no significant differences in ED50 values in OE and WT mice. PCPA pretreatment did not alter the hypothermic response to 8-OH-DPAT in mice. The hypothermic response to 8-OH-DPAT in mutant mice implies that postsynaptic 5-HT1A receptors could be involved in thermoregulatory function in mice. This assumption is further confirmed by the fact that 8-OH-DPAT-evoked thermal responses were not influenced by pretreatment with PCPA, most notably in transgenic mice overexpressing 5-HT1A receptors postsynaptically.

17.

Examining the interaction between in utero nicotine exposure and the D397N CHRNA5 nicotinic acetylcholine polymorphism (rs16969968) on alcohol intake in mice

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Despite the negative outcomes associated with smoking while pregnant, 15 – 30% of pregnant women in the U.S. smoke. Exposure to drugs of abuse in utero affect the developing brain and can lead to aberrant behaviors later in life. In particular, prenatal nicotine exposure has been associated with increased alcohol intake later in life. A SNP in the human CHRNA5 gene (rs16969968; D398N - D397N in mice) is reliably associated with increased risk for smoking, while also showing evidence of an association with alcohol use. Therefore, some offspring of pregnant smokers who carry the risk variant also carry the variant and are exposed to nicotine in utero. We explored this potential GxE interaction on later alcohol consumption using a mouse knock-in model with the D397N variant. Homozygous breeding dams for the risk (N397) and protective (D397) variants were given ad lib access to either plain drinking water or a 100 μg/ml
nicotine solution (with 0.2% saccharin for palatability) as their only fluid source from 30 days prior to mating, up through weaning. Offspring were allowed to mature undisturbed and then tested in a standard escalating alcohol concentration two-bottle choice procedure. Preliminary results suggest a GxE; nicotine exposed D397 mice drank more alcohol than their non-exposed counterparts, while nicotine exposed N397 mice drank less alcohol than their non-exposed counterparts. Results suggest that prenatal nicotine exposure has differential effects on later alcohol intake. Future work will assess whether these changes in drinking are related to changes in alcohol’s rewarding or aversive qualities.

1Institute for Behavioral Genetics, University of Colorado Boulder; Boulder, Colorado, USA. Funding Support: T32 AA007464; R21 DA040228

18.

The Heritability of Cocaine-Conditioned Avoidance Behavior

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Although millions of Americans are addicted to drugs of abuse, many more individuals are exposed but do not progress to compulsive use or addiction, possibly due to inherent protective factors. Addiction is strongly heritable, and many of these protective factors are likely to be under genetic influence. We and others have noted that some rats have particularly strong innate aversive responses to cocaine, that are strong enough to slow or block the transition from initial exploratory drug-taking to more regular sustained drug-seeking. Using inbred rats and the NIH Heterogeneous Stock (HS) rats, we found that innate avoidance responses to cocaine vary widely between strains, and respond rapidly to selective breeding. To rule out alternative explanations to our phenotype of interest, we have tested the animals on a battery of control tasks including
exploratory behavior, motivation for reward seeking, sensitivity to punishment from shock and cocaine behavioral economics. One of these control tasks, punishment resistance, is itself a defining feature of addictive behavior, as it measures the propensity to continue to seek rewards despite highly aversive outcomes we found that resistance to punishment is itself strongly heritable, and inherits independently of the aversion to cocaine.

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SLIT3 pathways regulate zebrafish nicotine preference and human smoking behavior

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Tobacco smoking is the leading preventable cause of death worldwide and places a heavy social and financial burden on society. Human smoking behavior is strongly heritable but the molecular mechanisms underlying nicotine preference are largely unknown. In a novel approach using a behavioral genetic screen of mutagenized zebrafish we demonstrate that nicotine preference is heritable in fish as in humans and identify loss-of-function mutations in the slit3 gene leading to increased nicotine preference. SLIT3 is a ligand for robo receptors, which have an established role in axon guidance and cell migration in the developing central nervous system. We show that fish heterozygous for loss-of-function mutations in slit3 have increased expression of the cholinergic receptor chrna5. Analysis of SLIT3 in humans identified genetic markers that predict level of cigarette consumption and likelihood of cessation, suggesting that evolutionarily conserved SLIT3 pathways acting through CHRNA5 regulate tobacco dependence in humans. The work I expect to present at the IBANGS conference are the cellular biological processes by which slit3 influences nicotine seeking behaviour. Preliminary analysis depicting TH labelled axons show altered axon pathfinding in slit3 loss of function mutants when compared with wild types, suggesting an effect in the guidance and outgrowth of dopaminergic axons that result in increased liking to nicotine. These findings suggest a role for SLIT:ROBO signaling in mechanisms underlying tobacco dependence and demonstrate the relevance of a zebrafish model in exploring complex human behaviors.

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Prenatal exposure to delta-9-tetrahydrocannabinol (THC) alters adolescent behavior and neurochemistry.

A.J. Elberger¹, K.M. Hamre¹, M.K. Mulligan², B.M. Moore, II³

With the recent spate of marijuana legalization, there will likely be exposure to individuals across their lifespan although little is known about the consequences of early exposure. In the present study, we examine whether prenatal exposure to delta-9-tetrahydrocannabinol (THC), the active ingredient in marijuana, has effects on behavior or neurochemistry at adolescence. C57BL/6J mice were exposed to either 2 or 6 mg/kg (Hi-THC) of THC in vehicle from embryonic day (E) 5-19 via IP injection; controls were given vehicle. Offspring were examined at adolescence. Depression was examined using the tail suspension test (TST); brain receptor expression was examined using immunohistochemistry. Many measures showed a dose-response effect with alterations observed primarily after Hi-THC. In the TST, Hi-THC mice exhibited depressive-like behaviors as shown by enhanced immobility. Prenatal exposure to THC also alters expression of in various neurotransmitter systems including serotonin in several brain regions, increased immunolabeling in the limbic system for the NR1 subunits of the NMDA receptor and the D1 dopamine receptor in the hippocampus. Cannabinoid receptor 1 expression was also increased in several brain regions including the amygdala, hippocampus, neocortex, and striatal system. These results demonstrate that prenatal exposure to Hi-THC can alter adolescent behavior and expression of specific components of neurotransmitter systems, providing a possible neuroanatomical basis for these behavioral alterations.

The influence of APOE status on functional network dynamics after traumatic brain injury

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A growing literature has used whole-brain fMRI analysis to examine the changes neural networks following traumatic brain injury (TBI) with recent analysis focusing on dynamic network properties over shorter windows of time. In behavioral studies of outcome after TBI, the apolipoprotein e4 allele (Apo-ε4) has been associated with poorer outcomes. We examine the relationship between Apo-ε4 status and network function in 14 individuals with moderate and severe TBI (+Apo-ε4 hetero or homozygous, n=7) and
14 age, education and Apoe matched HCs. Brain parcellation for network analysis involved independent components analysis of resting fMRI data and dynamic network analysis of the time series resulting in six distinct network “States”. The TBI sample was less likely overall (irrespective of Apoe status) to transition between network states and when considering Apo-ε4 status, the number of transitions between states was further diminished [Apo-ε4 + mean= 5.5 (sd=3.6); Apo-ε4- mean=8.9 (sd=3.4)]. In addition, the TBI sample revealed significantly lower frequency for State 5, a high-connectivity network state where the healthy adult sample showed the highest frequency. The reduced frequency in State 5 after TBI was significantly more pronounced in individuals with genetic risk [Apo-ε4+ mean frequency= 0.16, (sd=0.22); Apo-ε4- mean frequency= 0.43, (sd=0.28)]. Differences in brain networks were evident in the absence of any overt behavioral differences in cognitive performance. These data are consistent with a literature demonstrating that neurological disruption reduces network flexibility and restricted network dynamics may be more pronounced in individuals with TBI and genetic risk.

22.

Parent-of-origin effects and flexible covariance structure in guppy antipredator behaviors

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Whether trait covariation constrains the direction and rate of evolutionary change is a keenly debated topic in modern evolutionary biology. Non-independence among traits can restrict a population's response to selection depending on the genetic architecture. We used Trinidadian guppies (*Poecilia reticulata*) to explore behavioral covariation. Behavior, morphology, and life history have undergone parallel evolution in multiple river systems as high-predation ancestors colonized sites with low predation intensity. We generated genetically diverse families by creating intercross lines. We first crossed high- and low-predation populations from the same drainage, and then bred siblings of the resulting hybrid lines for two generations. We measured antipredator behaviors in intercross fish. For most measures, F2 hybrids showed intermediate antipredator behavior between parental populations. Cross direction yielded striking asymmetries in behaviors
of F2 fish, such that antipredator behaviors were more similar to the cross’s maternal ancestral population. Covariation among behaviors was also influenced by crossing such that behavioral correlations were weaker in F2 intercross fish compared to within-population lines. Further, several behavioral, life-history, and morphological phenotypes fell outside the range of the parent populations (transgressive inheritance) in the high-predation maternal and low-predation paternal cross direction. Our results suggest that parent-of-origin effects play a large role in the expression of suites of divergent traits in Trinidadian guppies. The rapid erosion of covariances suggests that behavioral covariation has a complex genetic basis and may not impose long-term constraints on adaptation in guppies.

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Effects of early postnatal ethanol exposure on medium spiny neuron morphology and synaptic protein expression

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The effects of ethanol exposure during development depend on the timing of exposure, since ethanol interrupts the specific brain development processes that are ongoing at the time of exposure, possibly resulting in Fetal Alcohol Spectrum Disorders. We investigated the effects of a single postnatal intoxication event on neuronal development in mice. We characterized the immediate effects of the ethanol exposure on the branching of the medium spiny neurons (MSNs) in the striatum, and also examined the long term effects throughout development. Animals were exposed to brief, high levels of ethanol during the early postnatal period (during
the most robust period of synaptogenesis). Mice were administered 2 doses of ethanol (2.5 g/kg) on postnatal day 5 (P5) two hours apart. Brains were removed and processed for Golgi-Cox staining in order to capture the cellular morphological response to the insult after 24 hours, after 1 or 5 months. These data find immediate increases in the branching morphology of MSNs as a consequence of neonatal exposure to ethanol, but differences do not persist into adolescence or adulthood. We also explored MSN spine number/type and levels of PSD-95 to determine whether active new synapses are being formed. The results characterize a novel response to ethanol exposure, as well as the dynamic nature of MSN dendritic branching. The caudate/putamen region is involved not only with the execution of complex motor skills, but importantly for alcohol research, these striatal areas can regulate aspects of long-term learning and addictive behavior.

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24.

Testing the Replicability of Mouse Phenotyping Experiments

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Phenotyping knockouts and other genetically-engineered mouse lines has become a central strategy for studying mammalian gene function. The utility of any findings, however, critically depends on the ability to replicate them in additional laboratories. We introduce a statistically based approach and a web-based application, which enable researchers to test the replicability of their results for several common procedures and phenotypic measures. The approach is a mixed-model ANOVA that models laboratories and their interaction with the genotype (GxL) as random effects. The application uses a database of previous results across several laboratories to estimate the GxL variability for each phenotype, and adjusts the ANOVA test accordingly in single-lab experiment results supplied by the user. Testing phenotyping experiment results using the application will be demonstrated. The application currently supports standard procedures and conditions of the International Mouse Phenome Consortium, and in the future will support additional procedures, based on data availability from multiple laboratories. Users of the application can also choose to submit their results, enriching the database with additional laboratories, genotypes, phenotypes and conditions, thus turning the application into a true community effort to ensure replicability of mouse phenotyping.

1Department of Statistics and Operation Research, Tel Aviv University, Tel Aviv, Israel. Funding Support: European Research Council grants PSARPS.
The First Molecular Mechanism for Personality Trait

Maryam Keshavarz, Rebecca Krebs-Wheaton, Diethard Tautz

Personality is defined as patterns of correlated behaviours that remain stable across time and contexts. Since the times of ancient Greeks, many studies have been done to discover the biological basis of personality and explain why individuals show stable personality, however so far no molecular mechanism has discovered that could explain this trait. In this study we revealed the first molecular mechanism for Personality trait which could nicely solve this puzzle. By using small RNA sequencing techniques, dd PCR, southern blot and computational methods, we showed copy number variation (CNV) in SNORD 115 and SNORD 116 which are small nuclear RNA, not only across mammals but also within population across individuals. Then we showed these variations lead to variation in expression of snoRNA target genes which are serotonin and GABA receptor in both wild and lab mice. Subsequently we demonstrated variation in serotonin and GABA receptor expression lead to variation in individual’s personality by using several behavioral tests such as elevated plus maze, open field, novel object and dark/light box over the course of 8 months. At next steps we studied inheritance of these snoRNA from parent to offspring. Results from mice family study revealed that during breeding, parents lose their snoRNA copy number pattern and each offspring showed different snoRNA copy number and subsequently different personality. As conclusion, we showed how CNV in SNORD 115 and 116 lead to having different personality and how variation in inheritance of these snoRNAs makes different personality within one family.
Forced Beach Test: A Novel Behavioral Despair Assay for the Zebrafish Model
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Zebrafish, a popular model system, is increasingly being used for behavioral genetic studies. Our lab is interested in how early life trauma can impact adult behaviors. However, assays for behavioral despair in adult zebrafish are lacking. The forced swim test is a behavioral despair test where rodents are placed in half-filled water tanks to examine their escape attempts. This test is useful in depression studies to assess the efficacy of antidepressant drugs in stressful situations. In this abstract, we present the forced beach test (FBT): a novel behavioral despair assay for small fish.

The FBT involves raising a false bottom to partially emerge zebrafish into shallow water and measuring their active efforts to find deeper water. An apparatus consisting of six transparent cylinders each containing an opaque moving cylinder with a porous bottom has been designed. When the apparatus is filled with water, the opaque cylinders can be moved up or down to modify swimming volume. The behavior of zebrafish as young as 10 weeks old will be recorded with camcorders for 8 minutes and analyzed manually, by two blinded scorers, to assess escape vs passive swimming behaviors.

A functional behavioral despair assay in small fish, like the zebrafish, will provide a fast, high throughput, and cost effective method for understanding the how genetics or developmental stressors can influence behavior including coping strategies that can be important in neuropsychiatric disease, like depression.
The role of \textit{DRD2} C957T and \textit{ANKK1} Taq1A polymorphisms in working memory performance: a systematic review and meta-analysis

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A recent systematic review and meta-analysis confirmed the role of the \textit{C957T} polymorphism of the dopamine receptor D\textsubscript{2} encoding \textit{DRD2} and the \textit{Taq1A} polymorphism of the neighbouring gene ankyrin repeat and kinase domain containing 1 (\textit{ANKK1}) in the development of schizophrenia (González-Castro et al., 2016). In addition, research in healthy adults suggests these functional gene variants alter dopaminergic signalling and may influence prefrontally-mediated cognitive functions such as working memory. As working memory deficits often precede the onset of schizophrenia (Fusar-Poli et al., 2012) a clear understanding of the current evidence of D\textsubscript{2} receptor functioning in the context of these specific gene variants and their role in working memory is needed. This study aims to systematically review the evidence for the association of \textit{DRD2 C957T} and \textit{ANKK1 Taq1A} polymorphisms with working memory performance in healthy adults. As part of a wider review, studies investigating the association between \textit{Taq1A} or \textit{C957T} polymorphisms and executive function in healthy adults were found using CINAHL, PsycARTICLES, PsycINFO and MEDLINE databases. Full-text scanning revealed 11 studies that focused on \textit{C957T} polymorphism and seven studies that investigated \textit{Taq1A} in the context of working memory. The most commonly used working memory tests included Spatial Working Memory (N=5), N-Back Task (N=5) and Digit-Span (N=3). Meta-analyses of this data will be presented separately for the \textit{Taq1A} and \textit{C957T} polymorphisms. This analysis will provide a much needed overview of the role of \textit{Taq1A} and \textit{C957T} polymorphisms in working memory ability and allow a greater understanding of their role in schizophrenia pathology.

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Susceptibility to psychosocial stress associates with morphological differences in the prefrontal cortex mitochondria of DBA/2NCrl and C57BL/6NCrl mice.

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Chronic social defeat stress (CSDS) increases social aversion in mice and is widely used as an animal model of anxiety and depression. Several studies in both human cohorts and animal models suggest the involvement of mitochondrial dysfunction in the etiology of these diseases. To determine whether mitochondrial morphology is affected by CSDS, and whether it is dependent on the genetic background, we investigated two mouse strains, C57BL/6NCrl (B6) and DBA/2NCrl (D2) that differ in stress reactivity and emotionality. We divided the animals to stress susceptible and resilient groups based on the social avoidance test after 10 days of CSDS. We then carried out transmission electron microscopy from the medial prefrontal cortex tissue of the mice. Mitochondrial cross-sections of B6 susceptible mice were significantly shorter compared to control B6 animals (p=0.004). D2 resilient animals had longer mitochondrial cross-sections in comparison with control (p=0.002) or susceptible D2 animals (p=0.001). We observed a significant strain effect in mitochondrial morphology. Control D2 mice had significantly larger number of mitochondrial cross-sections (p<0.001) that were significantly smaller in size (p<0.001) compared to B6 control mice. Interestingly, B6 susceptible animals did not differ from D2 controls while D2 resilient animals resembled B6 control and resilient animals. Our results suggest that chronic psychosocial stress affects mitochondrial morphology and that the effect depends both on the susceptibility phenotype and the genetic background. Therefore, CSDS provides a model to investigate chronic stress-induced changes and underlying mechanisms on mitochondrial function.

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ER stress markers in WFS1-deficient mice

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The WFS1 gene encodes an endoplasmic reticulum (ER) membrane-embedded protein and its loss-of-function mutations cause Wolfram syndrome (WS). WS is mainly characterized by diabetes mellitus and optic atrophy. Sensorineural deafness, diabetes insipidus, ataxia, urinary-tract atony, peripheral neuropathy and psychiatric illness may also be present. ER localization suggests that WFS1 protein has physiological functions in membrane trafficking, secretion, processing and/or regulation of ER calcium homeostasis. Disturbances or overloading of these functions induces ER stress responses, including apoptosis.

We extracted RNA from hippocampus, heart, liver, kidneys and pancreatic islets of WFS1-deficient mice, that have exon 8 disrupted, generated in our University. We measured gene expression of ER stress markers \textit{Atf6}\textsubscript{a}, \textit{Bip} (\textit{Hspa5}), \textit{Chop} and \textit{Wfs1} using TaqMan gene expression assays.

As expected \textit{Wfs1} was downregulated in all tissues. Upregulation of \textit{Bip} was statistically confirmed in each tissue type, except kidneys. The upregulation of \textit{Atf6}\textsubscript{a} was statistically significant in kidneys, but not in other tissues. The up- or downregulation of \textit{Chop} expression was not statistically confirmed in any of the tissues.

Although WFS1 is shown to be a novel component of the unfolded protein response and has an important function in maintaining homeostasis of the ER, the precise molecular mechanisms remain unknown. With further experiments, we would have a better understanding of WFS1 deficiency, ER stress and pathogenesis of the resulting diseases.

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Deep learning for mouse behavior recognition.

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Behavior is composed of a series of movements that are combined to produce an action in order to accomplish a goal. Generally, behavior is an output of the nervous system and is in response to environmental stimuli. The ability to automatically identify meaningful complex mouse behavior is an impediment for large scale experiments in the field of behavioral genetics and genomics. Deep learning are computational models that are composed of multiple processing layers to learn representations of data with multiple levels of abstraction. They represent state-of-the-art machine vision and machine learning. Here we use deep convolutional neural networks for general purpose mouse tracking and for recognition of mouse grooming behavior. For tracking, our trained neural network can track a mouse of any coat color in complex environment in light and dark conditions. It is a general-purpose mouse tracker that is highly adaptable to new environmental conditions. We are accurately able to detect grooming of mice and present a 45-strain survey of mouse grooming. Thus, deep learning with convolutional neural networks present a scalable and robust solution to alleviate the bottleneck in mouse behavior phenotyping.

31.

**Synaptic fusions in regulating sleep and wake activity in mice**

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More than a simple daily ritual, sleep is needed to maintain proper physiological functions like attention, learning and memory. Moreover, disruptions in the sleep and wake cycle have been linked to metabolic and psychiatric diseases. Using forward genetics and high throughput phenotyping methods in mice, we aim to identify genes that control sleep processes.

Here, we present a novel mutant mouse line carrying a missense mutation in vesicle-associated membrane protein 2, Vamp2. This mouse line (designated SLEEPY6) shows a number of sleep-related phenotypes including reduced total sleep time, increased activity and delayed sleep onset in the light phase. However, no associated deficits in circadian rhythm have been identified. These observations suggest that the mice show defects in sleep homeostasis. In addition to sleep abnormalities, SLEEPY6 mutants also lack nest building, freezing behaviour in fear conditioning, exploratory behaviours and some other innate behaviours. These phenotypes converge on anatomical changes in the vesicular transportation machinery we observed through imaging, electron microscopy and Golgi staining.

VAMP2 is part of the core SNARE (soluble N-ethylmaleimide-sensitive fusion attachment protein receptor) complex that is essential for chemical synaptic transmission. A spectrum of abnormal behaviours has been found in various SNARE mutant or knock-out lines including defects in sleep, circadian, and sensorimotor gating. Our findings in SLEEPY6 are consistent with these phenotypes. Investigations of the impact of the SLEEPY6 mutation in SNARE complex function at different neuronal types will help us further understand how synaptic fusion function mediates the cellular mechanisms of sleep and sleep homeostasis.
Stressor modality and recruitment of the hypothalamic-pituitary-adrenal (HPA) axis in rapid locomotor response to acute challenges in larval zebrafish

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Understanding the contribution of genetics and neural circuits on vertebrate-specific systemic stress response (SR) is a core interest in behavioral neuroscience. SR is mediated by the hypothalamic-pituitary-adrenal (HPA) axis in mammals (HPI (interrenal) axis in zebrafish) and results in neuroendocrine, immune, and behavioral responses. Although alterations in HPA axis activity are one of the most common pathophysiological changes in patients with depression, it has been challenging to identify the genetic modifiers of SR due to individual genetic variations and environmental interactions. Larval zebrafish show a rapid locomotor response to acute challenges, and can be used to independently dissect both genetic and environmental contributions. We hypothesize that, based on the types of challenges, a subset of the observed rapid locomotor response depends on the HPI axis and is a reliable readout for SR. Confirming our hypothesis, an acute hyperosmotic challenge (100 mM NaCl) results in a rapid increase in locomotion that is dependent on mc2r (adrenocorticotropic hormone receptor). Abrupt changes in light conditions (45-seconds of white light stimulation to dark-adapted fish) leads to rapid increase in locomotion, which depends on mc2r or nr3c1 (glucocorticoid receptor). However, an acute nicotine application (20 μM nicotine) increases locomotion independent of the HPI axis. Our data demonstrates that rapid locomotor response recruits the HPI axis differentially depending on stressor modalities. Further, the rapid locomotor assay can be used as a screening platform to identify genetic modifiers of the HPI axis to uncover new therapeutic targets for stress-aggravated disorders.
Variant in stress-related genes may contribute to maintaining long-term abstinence in former addicted subjects who are not treated with opioid agonist

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Opiates addiction requires long-term management. Only a small percentage of individuals meeting criteria for heroin dependence are able to maintain long-term abstinence without pharmacological treatment. Stress is a critical factor affecting both the development of addiction and relapse. There is a high inter-individual variability in the response to stress that is influenced by a combination of genetic and non-genetic factors. This study was designed to assess whether genetic factors in stress-related genes are associated with long-term abstinence from heroin in subjects that are not treated with medication. Genotype frequencies of 118 polymorphisms in 30 genes were compared between former opiate-dependent individuals either in long-term abstinence without agonist treatment (MF, n = 129) or in long-term methadone maintenance treatment (MMT, n = 923). All subjects have predominantly European/Middle Eastern ancestry. Three SNPs (rs1500 in the CRH binding protein gene, CRHBP; rs4234955, in the intergenic region of NPY1R and NPY5R; and rs10482672 in the glucocorticoid receptor gene, NR3C1) showed significant associations with long-term abstinence under the dominant model that remained significant after permutation corrections. The signal for the CRHBP SNP remained significant after permutation correction accounting for ancestry substructure. This study provides preliminary evidence that genetic variability may contribute to the distinct ability of particular subjects to remain abstinent from heroin for a long period without pharmacological treatment, possibly indicating higher resilience to stress. Identification of factors that contribute to long-
term abstinence from heroin is an important step toward a better understanding and treatment of drug addiction and relapse.

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Cognitive flexibility/stability using touchscreen technology and simultaneous severity assessment in mice

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The possibility to adjust behavior due to novel demands (flexibility) or to remain on a behavioral plan despite distraction (stability) are key features of executive functioning. An antagonistical relationship between both functions has been suggested by neurocomputational models derived theories, which were supported by findings of a human paradigm study aiming to analyze the neural correlates.

We translated this procedure into the mouse-suited touchscreen-based task and investigated conceptual predictions of individual predisposition towards stability/flexibility and a dichotomous relationship of both functions. Therefore, mice were trained on a two-choice operant discrimination task and had to respond on touch fields dependent on the position of a visual cue. The results confirm the challenged predictions and indicate cross-species translational potential of the task.

The training effect on stress levels and hippocampal activity was analyzed in a simultaneous severity assessment study. After an adaptation of initial corticosterone level elevation, BDNF data indicate rather a eustress-like response attributable to daily training.

Further CACNA1C KO mice (risk gene for psychiatric disorders) were tested for cognitive alterations. Interestingly, they revealed a deficit in cue-association learning, but demonstrated an unintended strategy for reward optimization.

The newly implemented paradigm for mice offers new opportunities for investigation of underlying mechanisms of cognitive stability/flexibility on the preclinical side of translational research with high validity.
Alpha-Adrenergic Modulation of Ethanol Intake in C3H/HeJ mice.
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Anxiety disorders and alcohol use disorders are highly comorbid, and excessive noradrenergic signaling may contribute to both. Thus, anxiolytic beta- and alpha1-adrenergic receptor antagonists can reduce alcohol consumption, while anxiogenic alpha2 antagonists can trigger relapse-like behavior in animal models. Nevertheless, the conditions under which such effects are seen are not fully specified. In the current study, the alpha1 antagonist, prazosin, and the alpha2 antagonist, yohimbine, were tested for their ability to modulate voluntary ethanol intake under both continuous and intermittent ethanol access schedules in male C3H/HeJ mice. Neither drug affected ethanol intake under initial continuous access conditions, but yohimbine potentiated the alcohol deprivation effect under intermittent ethanol access. When continuous ethanol access was later restored, yohimbine potentiated and prazosin reduced ethanol intake. These results indicate that the ability of alpha-adrenergic drugs to modulate voluntary ethanol consumption depends on both ethanol access schedule and prior drinking history. Our findings may inform future clinical application of adrenergic drugs in the management of alcohol use disorders, especially when comorbid with anxiety.
Title
Prophylactic ketamine reduces fear expression but does not facilitate extinction

Authors
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Abstract
Ketamine, an N-methyl-D-aspartate (NMDA) glutamate antagonist, has been reported to be an efficacious antidepressant for depression and posttraumatic stress disorder, and most recently, to be a prophylactic against stress-induced depressive-like behavior. It remains unknown, however, when ketamine should be administered relative to a stressor or depressive episode in order to maximize its beneficial effects. Furthermore, it is unknown if ketamine can be prophylactic against subsequent episodes. Here, we systematically tested the utility of ketamine relative to a fear experience in order to determine the best interval for ketamine to be administered in order to reduce fear or act as a prophylactic against subsequent aversive episodes. Using a 3-shock contextual fear conditioning (CFC) paradigm, we tested if ketamine could alter how 129SvEv mice respond to fear. Mice were administered a single dose of saline or ketamine (30 mg kg⁻¹) at varying time points before or after CFC, extinction, or reinstatement. Mice administered prophylactic ketamine 1 week before, but not 1 month before, CFC training exhibited reduced freezing behavior when compared with mice administered saline. In contrast, ketamine administration following CFC or during extinction did not alter subsequent fear expression. Interestingly, mice administered ketamine 1 h before CFC exhibited increased freezing behavior when compared with mice administered saline. These data indicate that ketamine can diminish
the fear response when given as a prophylactic, but not when given immediately before or after an aversive episode. Therefore, ketamine may be most useful if administered in a vaccine-like fashion to protect against fear-inducing stimuli.

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37.

**A broad analysis of anxiety-like behavior in Neto1 knock-out mice**

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Ionotropic glutamate receptors mediate the majority of excitatory neurotransmission in the central nervous system. This class of receptors includes N-methyl-D-aspartate receptors (NMDAR) and kainate receptors (KAR), which interact with the auxiliary protein Neto1 at the post-synaptic density. Considering that Neto1 is expressed in brain regions involved in the regulation of anxiety coupled with the fact that both NMDAR and KAR regulate anxiety, we hypothesized that Neto1 may modulate anxiety-like behavior in mice. We tested this hypothesis by carrying out a comprehensive behavioral analysis of Neto1 knock-out, heterozygous, and wild-type mice, both males and females that were 8 weeks old at the beginning of the testing. We did not observe differences between the genotypes in anxiety-related tests based on approach-avoidance conflict (elevated plus maze, marble burying, novelty-suppressed feeding, open field, and light/dark box tests) or in home cage locomotor activity. Neither did we detect differences in cued fear conditioning or fear extinction between the genotypes. We are currently carrying out contextual fear conditioning, known to involve the hippocampus, where Neto1 is especially highly expressed. In conclusion, our results demonstrate that lack of Neto1 gene does not affect baseline anxiety-like behavior in mice. Based on these results we cannot exclude a possible role of Neto1 in stress-induced anxiety-like behavior; this requires further investigation.

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Neuronal activation associated with genetically-determined methamphetamine consumption differences and drug-related thermal regulation disruptions
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Mice selectively bred for high and low methamphetamine (MA) drinking (MAHDR; MALDR) exhibit MA-related behavioral differences, and differences in thermal response to MA. To map potential brain regions underlying these differences, we examined c-Fos expression after saline, acute and chronic MA exposure. Mice (12/group) were injected with saline or MA once a day for 6 days. All mice received saline on days 1-3. Separate groups then received saline (days 4-6); saline (days 4-5) then 2 mg/kg MA (day 6); or MA (days 4-6). Brains were removed, sectioned, and stained for c-Fos. MAHDR mice had significantly higher overall expression in the nucleus accumbens core (F[1,57]=5.87, p=0.02), and significantly lower expression in the dorsomedial hypothalamus (DMH) after acute MA exposure, compared to MALDR mice (F[2,58]=6.61, p<0.01). The role of the DMH in body temperature regulation led us to explore temperature changes in response to additional drugs of abuse. Mice were injected with morphine (15 or 30mg/kg), cocaine (15 or 30 mg/kg), MDMA (2.5 or 5mg/kg), or saline, and their temperatures were tracked over 3 hours. There was an effect of sex on body temperature response to morphine (F[12,288]=1.85, p=0.04, sex x time x dose), but no significant difference between the lines in temperature responses to morphine or cocaine. However, the MALDR line exhibited hypothermia in response to MDMA that was absent in the MAHDR line (F[12,288]=1.8, p=0.04, line x dose x time). Additional data are needed to link DMH activation with differences in thermal responses unique to amphetamine-like substances in these lines.
Connecting genes to behavior in Alzheimer’s disease – utilizing mouse genetic diversity.

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Alzheimer’s disease (AD) pathology is characterized by accumulation of beta amyloid plaques, neurofibrillary tangles of tau (NFT), marked neuroinflammation and widespread neuronal loss. Unlike in human AD patients, mouse models show minimal neurodegeneration. A major
difference, often overlooked, between existing mouse models of AD and the human population is the level of genetic diversity, as current models are almost exclusively developed in a single mouse strain: C57BL/6J (B6). This is equivalent to studying AD in a single patient and has severely limited our ability to develop effective therapies for AD, as well as thoroughly interrogate the relationship between associated risk behaviors such as sleep pattern, activity and anxiety. To address this, we have created a genetically diverse panel of inbred mouse models by backcrossing the familial mutant forms of amyloid precursor protein (APP\textsuperscript{swe}) and presenilin 1 (PS1\textsuperscript{dE9}) from B6 to the founder strains of the Collaborative Cross. This is the first time that the wild-derived strains – CAST/EiJ, PWK/PhJ and WSB/EiJ – have ever been used to study AD. Our data indicate that these strains present significant variation in AD relevant phenotypes including metabolic dysfunction, cognitive decline, tau pathology and neuronal cell loss in AD-susceptible brain regions. Importantly, these strains also show significant variation in associated AD-risk behaviors. Our data lay the foundation for further genetic studies to identify novel therapeutic targets and improve translatability for preclinical trials.

Putative involvement of Lrrk2 gene on the inflexible ethanol preference behavior in adult Zebrafish.

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In a population of adult Zebrafish submitted to the Conditioned Place Preference behavioral (CPP) test, different phenotypes regarding ethanol preference were observed. For this test were used animals that never had contact with ethanol as a group of animals that went through a chronic exposure of 16 days. The preference was determined in two observations, before and after a withdrawal period. In both, the never exposed and the chronic exposure group, four phenotypes were distinguished. The first phenotype, Inflexible, are animals that showed preference for alcohol at the first observation and maintained after 16 days of abstinence. The second, Abdication, showed a preference on the first observation, but after withdrawal they no longer preferred. The third, Negative reinforcement, only prefers alcohol after abstinence, and the fourth and last, Light phenotype, had no preference in any of the observations. Given the phenotypes found and previous data from our laboratory with mice linking the Lrrk2 gene to the ethanol compulsive consumption, the objective of this work is to verify if the same type of association occurs in Zebrafish. For this, initially, the two extreme groups (Inflexible and Light) were selected for the quantification of transcripts of the Lrrk2 gene by qPCR in Zebrafish brain samples. It was observed that Lrrk2 is up-regulated in the Inflexible group in relation to the Light, supporting the hypothesis that this gene may be involved in the development and/or maintenance of alcohol use disorders.
Genome-wide mapping of conditioned fear in the Diversity Outbred mouse population

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Mice offer a powerful tool for elucidating the genetic basis of traits relevant to anxiety disorders; yet conventional experimental crosses have only identified large chromosomal regions rather than specific genes. Recent advances have led to genetically diverse, highly recombinant mouse populations. We have taken advantage of the newly developed Diversity Outbred (DO) mice to map narrow QTLs associated with conditioned fear (CF). We phenotyped 587 DO mice for the acquisition, extinction, and renewal of CF using a three-day paradigm. We genotyped a subset of these mice at ~150k markers across the genome and performed high precision QTL mapping using the R program DOQTL. A one-way repeated measures ANOVA found a significant increase in freezing following each tone-shock pairing during acquisition, \( F_{1.8, 892.8} = 799.5, p < 0.0001; \eta_p^2 = 0.612 \), demonstrating the ability to learn to associate the tone and foot-shock. Freezing behavior in response to the tone significantly decreased across trial-blocks during extinction training \( F_{6.2, 3619.4} = 145.6, p < 0.0001; \eta_p^2 = 0.199 \) suggesting mice were able to successfully extinguish the fearful association over time. On the renewal test, mice displayed less freezing relative to the first trial-block of extinction training \( t(586) = 13.7, p < 0.0001 \). QTL analyses identified numerous suggestive and significant QTLs associated with CF on chromosomes 2, 3, 7, and 12. With the inclusion of RNA-Seq we will be able to apply a systems genetic strategy to construct the network of correlations that exist between DNA sequence, gene expression values and CF.
Environmental enrichment in the form of nesting material partially rescues the neurobehavioral abnormalities of a genetic mouse model of Fragile X syndrome.

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Environmental enrichment (EE) is known to induce marked effects on the brain and behavior of laboratory mice, including genetically modified lines modelling mental disorders. The growing interest in research on EE has therefore multiple reasons: EE is a valuable tool to study neurobehavioral plasticity, with an emphasis on gene-environment interactions and direct implications for the design of non-pharmacological therapies. EE is also of crucial importance for the welfare of laboratory animals, and has become a mandatory procedure for mouse husbandry in several countries. Nesting material is commonly used as EE for laboratory mice, since it is easy to implement as cage enrichment from the practical point of view and it has ethological relevance, e.g., allowing animals to perform the natural activity of nest building and to hide from the experimenters’ sight. Here, we have investigated whether exposure to nesting material starting from birth would affect the neurobehavioral phenotype of the Fmr1-KO mouse model for Fragile X syndrome (FXS), a major developmental disorder. Enrichment partially rescued the FXS-like phenotype of KO mice both at infancy and at adulthood, and its effects differed between sexes. Furthermore, enrichment altered the levels of tri-methylation of H3K27, i.e., a marker of brain plasticity, in several brain areas. Our results demonstrate that nesting material is a powerful enrichment tool, exerting marked neurobehavioral effects in a genetic mouse model of FXS. Thus the role of gene-environment interactions should be taken into consideration carefully before implementing nesting material routinely as EE for mouse models of neurodevelopmental disorders.

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Multifaceted cognitive ability in selected mouse line

OV Perepelkina, AYu Tarassova, IG Lilp, II Poletaeva

Different types of learning, logic problem solving, spatial orientation, neophylia, attention—all these behavioral categories are included in the term “animal cognition”. The selection of mice for the definite cognitive trait – the ability to solve the “extrapolation task” was not successful in the “straightforward” sense, as it was in cases of selection for different learning skills. “Extrapolation” task implies ability of an animal to follow (mentally) the direction of food movement after it disappears from its view and then to locate the food reward. The variability of correct choices proportion along selection generations was found both in the non-random performance evaluation and in comparison with the scores of non-selected control mouse population. At the same time the performance of mice from the selected line was persistently higher in another cognitive problem solving – the puzzle box (burrowing) task, based on innate avoidance of brightly lit space. These data could be explained if we suggest that brain network program which realizes the solution of extrapolation task (ie providing the respective executive function) is much more complicated, than that in case of burrowing task. This complexity could be due to recent memory, attention, reaction to novelty domains each of which could possess its own signaling pathways and brain substrates. Although, the selection for the cognitive trait could be regarded as successful. Biology Department, Moscow State University, Moscow, RFBR #16-04, Education Ministry program № N NIOKTR AAA-A16-116021660055-1, RUSSIA
Rodent audiogenic epilepsy (AE) is the complicated polygenic trait, and several selected for AE proneness were described for rats, mice and hamsters. The neurophysiology of AE was analyzed in details, while its “comorbidity” with other pathological states, as well as the developmental regulation are not clear. In rats of Krushinsky-Molodkina (KM) inbred strain AE seizures develop in 100% of individuals with minimal latency (5-7 srec for tonic seizure stage). Aiming to define the genetics of AE two new rat strains were bred recently (from hybrids of KM and non-AE-prone Wistar) using two back-crossed to KM parental strain. One of them (“0” strain) was selected for lack of AE seizure, while another (“4” strain) - for intense AE seizure. The genetic analysis data of the first hybrid generations permitted to confirm the recessive polygenic nature of AE genetic determination (describing at least two genes-modifiers). This panel of strains permitted to analyze the influence of maternal methyl-enriched diet (which decreased AE in “4” strain, but not in KM), as well as to describe the detailed pattern of comorbid and non-comorbid traits (i.e. anxiety and depression), which prove that the comorbidity in this seizure model depends largely on the influence of the genetic background (as “0”, “4” and KM are more closely related than Wistar). Biology Department, Moscow State University, Moscow, Russia. Country Funding Support: RFBR (grant # 15-04-1732), and The Program N AAA-A16-11602166005-1.
Transcriptome analysis reveals common networks of alcohol-related genes in mice and men: focus on neuronal plasticity in the extended amygdala

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Alcohol Use Disorder (AUD) is a complex psychiatric disorder with strong genetic and environmental risk factors. One risk factor for developing AUD is binge drinking. High Drinking in the Dark mice (HDID) have been selectively bred for attaining high blood alcohol concentrations (BAC) after a 4-hour drinking session during which a single bottle containing 20% ethanol is available and serve as a genetic model of binge drinking. To discover molecular mechanisms underlying the genetic predisposition to binge drinking, we characterized gene expression in 7 brain regions across the addiction neurocircuit, precisely excised using laser capture microdissection from male, ethanol-naive HDID and control mice using microarrays. Selective breeding for intoxicating BACs resulted in global changes in brain gene expression. A network approach partitioned the enormous diversity of transcriptional responses to genetic selection into several modules of correlated genes, which represented well-characterized functional groups and molecular pathways in specific cell types. Comparing gene coexpression modules between the mouse model of binge drinking and postmortem brains of human alcoholics revealed a meta-network of highly overlapping modules, suggesting that gene regulation is conserved across species. Based on this comparison, we hypothesized that interplay between glutamatergic and dopaminergic signaling in the extended amygdala plays a central role in regulating ethanol consumption across species. Whole cell voltage clamp recordings from nucleus accumbens shell neurons projecting to the ventral tegmental area partially validated this hypothesis, showing differential ethanol-induced plasticity in HDID and control mice. Overall, our findings imply mechanistic links between the genetic predisposition to binge drinking in an animal model and AUD in humans, proposing possible points of intervention to prevent and/or treat AUD.

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Evidence for an epistatic effect of \textit{Oprm1} and \textit{Taar1} on methamphetamine-induced hypothermia

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The trace amine-associated receptor 1 (\textit{Taar1}) gene impacts methamphetamine (MA)-induced hypothermia, a physiological effect associated with low MA intake. In addition, the mu-opioid receptor gene, \textit{Oprm1}, is regulated by a transcription factor network underlying risk for MA intake. The current studies further investigated the influence of these genes on body temperature after MA administration. In Experiment 1, C57BL/6J (B6) mice and vendor-specific DBA/2 (D2) mice that have different \textit{Taar1} allele types were tested for MA-induced hypothermia (2 mg/kg). In Experiment 2, B6xD2 recombinant inbred (BXD RI) strain mice with different \textit{Taar1}/\textit{Oprm1} allele combinations were similarly tested. B6 and D2/NTac mice have the same B6-like-\textit{Taar1} allele, whereas D2/J mice have an alternative allele that codes for a non-functional receptor. B6 and D2/NTac mice exhibited MA-induced hypothermia that was absent in D2/J mice ($F[12,786]=10$, $p<0.0001$, strain x treatment x time interaction). In the BXD RI strains, mice with the B6-\textit{Taar1} allele displayed MA-induced hypothermia that was absent in mice with the D2/J-\textit{Taar1} allele ($F[6,828]=27.3$, $p<0.0001$, \textit{Taar1} allele x treatment x time interaction). There was also a significant \textit{Oprm1} allele x \textit{Taar1} allele x treatment x time interaction ($F[6,780]=2.8,p=0.01$), with mice that were D2-\textit{Oprm1}/B6-\textit{Taar1} displaying the greatest MA-induced hypothermia compared to the all other \textit{Oprm1}/\textit{Taar1} groups, and B6-\textit{Oprm1}/B6-\textit{Taar1} mice displaying greater hypothermia than B6-\textit{Oprm1}/D2-\textit{Taar1} and D2-\textit{Oprm1}/D2-\textit{Taar1} groups. These data confirm that functional TAAR1 is required for MA-induced hypothermia and suggest an epistatic \textit{Oprm1xTaar1} interaction in sensitivity to MA-induced hypothermia. Additional data are needed to identify gene-specific mechanisms associated with sensitivity to MA-induced hypothermia.
LED Green light treatment improves learning and memory in mice

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Phototherapy has been shown to be effective for a variety of mental diseases. For example, blue light treatment can effectively improve learning and memory functions in Alzheimer’s patients. However, the known retinal damage effect of blue light stimulation limits its application. In this study, we investigated the effect of LED green light (530nm) treatment in the regulation of whisker-dependent learning and memory in mice. We found that green light treatment could improve whisker dependent learning and memory via BDNF-TrkB pathway.

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48. Candidate molecular mechanisms linking \textit{Hnrph1} polymorphisms with psychostimulant and opioid-induced behaviors

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\textit{Hnrph1} (heterogeneous nuclear ribonucleoprotein H1) was identified and validated by our lab as a quantitative trait gene (QTG) underlying sensitivity to the locomotor stimulant, rewarding, and reinforcing properties of methamphetamine (MA). Transcriptome and spliceome analysis of the
striatum in congenic mice capturing a 112 kb region containing Hnrnph1 polymorphisms between C57BL/6J and DBA/2J inbred mouse strains and in Hnrnph1+/− mice were conducted to identify candidate mechanisms of Hnrnph1 regulation of MA. The congenic allele showed decreased usage of exon 3 of Hnrnph1 that was located near a single SNP within the 5’UTR and two other nearby intronic SNPs. In addition, 112 kb congenic mice showed increased usage of exon 1 of Ppp3ca which codes for a subunit of calcineurin. Interestingly, human splice variants of PPP3CA have been associated with drug addiction vulnerability. These results provide important mechanistic links between Hnrnph1 splice target events and the addictions. In a separate series of studies, neuronal localization of hnRNP H following depolarization and dopamine receptor activation was examined in primary rat cortical neurons. D1 receptor stimulation induced an increase in nuclear intensity of hnRNP H, which suggests that post-synaptic dopaminergic signaling modulates hnRNP H function. Finally, to potentially extend the role of Hnrnph1 in behavioral sensitivity to opioids, locomotor stimulant and rewarding response to fentanyl (FENT), a mu opioid receptor (MOR) agonist, were characterized. Similar to MA, Hnrnph1+/− mice also showed a reduced sensitivity to FENT. However, these mice did not show differential response to the analgesic effect of FENT, which suggests a selective role of Hnrnph1 in neurodevelopment of the brain reward circuitry. Interestingly, hnRNP H has previously been shown to regulate the expression of Oprm1, which codes for MOR, through post-translational repression and alternative splicing.

49.

Enhanced empathic fear with thalamocortical dysrhythmia in phospholipase-C beta4 deficient mice

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Empathy is an important capacity to recognize and share emotions with others. The anterior cingulate cortex (ACC) and the mediodorsal thalamus (MD) were shown to be activated when people engage in empathy, and to be involved in acquisition of observational fear in mice. Here, we demonstrate that a distinct thalamocortical sub-circuit, reciprocal excitatory projections between the lateral part of MD (MDL) and the ACC is critical for observational fear, and a dysrhythmia of this circuit induced by a mutation of phospholipase C beta4 (Plcb4) leads to enhanced observational fear. Mice with a specific deletion of Plcb4 in the MDL-ACC projection showed a marked increase in observational fear. ACC projecting MDL neurons lacking Plcb4 in slices showed enhanced burst firing and in vivo recording in mice with MD-restricted deletion revealed increased neuronal activities in the ACC and the MD, and an augmented theta synchrony between the two regions. We further confirmed the enhanced excitability of MD neurons in the Plcb4-deficient mice was dependent on T-type Ca^{2+} channel activity: an MD-restricted deletion of Cav3.1 T-type Ca^{2+} channel suppressed the enhanced observational fear response of the Plcb4 mutant. Optogenetic activation of the MD input to the ACC evoked an elevation of vicarious freezing, mimicking the Plcb4 mutation, whereas suppression of this MDL-ACC connectivity in either direction impaired observational fear. Taken together, our data indicate the reciprocal MD-ACC pathway constitutes an essential circuit for observational fear, and an aberrant rhythmicity in this circuit leads to dysfunction of cognitive processing for empathic fear in mice.

50.

GABA in basolateral amygdala mediates the effects of stress on enhanced reacquisition of nicotine self-administration

**Burt M Sharp**

Chronic stress is a major cause of human relapse to smoked cigarettes. In a rat model, we have shown that repeated stress, during abstinence from nicotine self-administration (SA), enhances the reacquisition (REAQ) of nicotine SA by increasing the motivation for and intake of nicotine.
Basolateral amygdala (BLA) and its connections to nucleus accumbens core (NAcc) are necessary for this stress-induced amplification of nicotine taking. We have reported that reversible, bilateral inactivation of BLA eliminated stress-enhanced nicotine SA without affecting the underlying level of nicotine SA observed during REAQ in non-stressed rats. We hypothesize that disinhibition of glutamatergic principal neurons projecting to NAcc, due to reduced BLA GABAergic inhibition of these neurons, is required for enhanced REAQ of nicotine SA. This hypothesis was tested by microinfusing two different positive allosteric modulators of GABAA receptors (R) into BLA bilaterally just prior to testing REAQ behavior. TP003, a relatively subtype-selective modulator of GABAA-R that is anxiolytic, apparently via α3 subunits in rodent and non-human primate models, completely abolished the stress-enhanced component of nicotine SA during REAQ, whereas post-abstinence nicotine SA was unaffected. A more selective modulator that interacts only with α2/α3 subunits, NS 16085 (Saniona, Inc.), is under study; preliminary observations suggest similar activity. In summary, repeated stress during abstinence appears to reduce BLA GABAergic inhibition of principal output neurons, enhancing nicotine SA and motivation to take nicotine during REAQ through connections from BLA to NAcc.

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51.

PhenoMiner: A unique resource for mining and analyzing quantitative behavioral phenotype data in the rat

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RGD (http://rgd.mcw.edu) is the premier online resource for rat genetic, genomic and phenotypic data, offering a large body of cross-species data and innovative software tools to facilitate the search for appropriate models for human diseases and behavioral conditions such as alcoholism and anxiety disorders. Among these, the PhenoMiner tool stands out as a unique resource for mining and analyzing quantitative rat phenotype data. PhenoMiner uses controlled vocabularies to standardize the representation of the trait being assessed, the specific measurements made, methods used, experimental conditions under which the measurements were made and rat strains used. In addition to providing a more complete picture of the contributing factors influencing each measurement value, this standardization allows users to compare results across strains, across conditions and across studies. PhenoMiner data is accessed using a flexible search tool which allows researchers to filter on any of four parameters in any order. Once a researcher has selected their filters, the result set can be seen in a chart or tabular view. In addition, the result set can be downloaded to be saved and/or used in other tools. Currently, work is underway to expand and refine the representation of behavioral phenotype data in the PhenoMiner tool. To this end, RGD curators are interested in getting feedback (positive or negative) from behavioral researchers about the representation of behavior and movement in PhenoMiner. Comments and suggestions will be welcomed.

52.

Are the effects of early-life stress on cocaine effects in adulthood due to long-term down regulation of GABRA2 expression?

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Exposure of mouse pups to early life stress (ELS) during postnatal days 2 – 9 decreases adult levels of GABA_A receptor α2 subunits in ventral striatum, and impairs behavioural sensitisation
to cocaine. Similar behavioural effects occur following targeted deletion of α2 subunits. We now imposed ELS on litters from parent mice heterozygous for α2 deletion, giving pups homozygous for α2 deletion (KO), heterozygotes (HT), or wildtypes (WT).

Acute locomotor responses to cocaine (10 mg/kg, i.p.) were greater in ELS vs. non-stressed mice. Locomotor activation was also greater in KO animals, but independently of drug or ELS condition. HT mice were intermediate, but did not differ statistically from either WT or KO. Repeated cocaine administration resulted in sensitisation, which was decreased by ELS. The degree of sensitisation depended upon genotype, confirming the previously reported absence of sensitisation in KO mice, but revealing that HT mice exhibit normal sensitisation. Relative to the equivalent non-stressed groups, exposure to ELS did not further affect the degree of sensitisation in KO, but abolished the development of sensitisation in HT mice.

Conditioned activity was deduced from increased locomotion in the test environment previously paired with cocaine. Conditioned activity was greater in ELS animals, but was independent of genotype. These finding are consistent with ELS mimicking deletion of α2 in its effects on behavioural sensitisation to cocaine, but ELS effects on cocaine-conditioned activity appear independent of α2.

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53.

Predictors of women’s eating problems: The moderating role of triallelic serotonin transporter and serotonin receptor 2A genotypes on the effect of objectification

GA Sullivan1, SJ Gervais1, RL Brock1, & SF Stoltenberg1
This study investigates individual differences in the development of eating problems. We test a model based on objectification theory in which previous experiences of objectification predict eating problems through body shame, and include triallelic serotonin transporter (SLC6A4) genotypes as a moderator. Five hundred thirty-nine undergraduate women (78.66% White, mean age = 20.28) participated. Participants came into the lab, gave informed consent, completed questionnaires, and donated buccal cells for genetic analyses of 5-HTTLPR and rs25531. Past experience of sexual objectification was measured using the Interpersonal Sexual Objectification Scale (Kozee et al., 2007), body shame was measured using a subscale of the Objectified Body Consciousness Scale (adapted from McKinley & Hyde, 1996), and eating attitudes were assessed using the 26-item version of the Eating Attitudes Test (EAT-26; Garner et al., 1982). The association between experience of objectification and eating problems was mediated by body shame, and the association between body shame and eating problems was moderated by SLC6A4 genotype, with a stronger association for L_A homozygotes (L’/L’). Body shame is a better predictor of eating problems for women with the L’/L’ genotype than S or G allele carriers (S’/__). This adds to evidence that risk factors for eating disorders should be weighted differently based on genetic differences.

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Preference: a) I prefer to present a poster, but would be happy to deliver an oral presentation, alternatively.

54.

The critical role of micronutrients in neurodevelopment: short- and long-term behavioral outcome in a Selenium-deficient rat model
Tartaglione AM¹,², Scalfari A¹, Attorri L³, Di Biase A³, Minghetti L⁴ and Calamandrei G¹
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Research in animal models and human population shows that some essential elements such as selenium (Se) are particularly important during early stages of life to support rapidly maturation of cognitive functions. Thus, we characterized the neurobehavioral effects of three diets with different Se content administered since the pre-conception stage up to adulthood. In order to increase the translational value of our rat model we chose to perform a longitudinal assessment (since the very early neonatal stage to adulthood), by selecting different behavioral domains to evidence even subtle functional changes attributable to Se deficiency.

Adult females were assigned to one of three experimental groups based on different Se content of diet given (Se-deficient diet, Se-suboptimal diet, and Se-optimal diet). Females were exposed to a specific diet one month prior to mating through pregnancy and lactation. At weaning, offspring were fed the same diet as their respective dams until adulthood and completion of the behavioral assessment.

Offspring of both sexes were assessed for somatic growth, spontaneous locomotion, ultrasonic vocalizations and nest-odour recognition from postnatal day 4 to 12. At the juvenile and adult stage rats underwent tests assessing motor/explorative, emotional and cognitive domains. On the basis of the still scarce evidence in the literature, we expect to identify early behavioral changes that may be predictive of later deficits. The results of our research will be of potential high significance to elucidate the possible outcomes due to an unbalanced diet, to promote optimal brain development and possible intervention under adverse environmental challenges.

55.

Characterization of the ventral tegmental area in socially monogamous prairie voles.

J.A. Temple¹, K. Gordon², Z.R. Donaldson¹,²
Pair bonds are long lasting social attachments characterized by an affiliative preference for a particular individual. Pair bonds are a vital part of the human experience and a fundamental aspect of human nature that has been extensively implicated in modulating health and well-being. Despite the relevance of these attachments, we lack an understanding of the neural circuitry that contributes to pair bonding. Largely, this is due to a paucity of appropriate animal models for studying this trait. Only 2-5% of mammals are monogamous and display pair bonding behaviors, and laboratory rats and mice do not fall in this category. Prairie voles are socially monogamous rodent species characterized by selective mating, mate guarding, and bi-paternal care. Pair bonding in this species is thought to engage parts of the brain that process natural reward. In particular dopamine (DA) signaling in nucleus accumbens (NAcc) is necessary for pair bonds. I hypothesis that the ventral tegmental area (VTA) serves as a critical source of DA in the NAcc in the context of partner preference. Firstly, I am characterizing the neurons that project from VTA to NAcc in prairie voles. Studies in other species indicate that the VTA is comprised of several cell types, but that the NAcc is preferentially innervated by more lateral, DA-expressing cells from this region. Thus, we are currently investigating whether this is also true in voles by using both retrograde tracing techniques and immunohistochemistry. These results will provide a characterization of these projections as the basis for future functional studies.

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56.

Unpredictable chronic mild stress: behavioral responses, neurotransmitter systems and effect of regional deficit in serotonin synthesis
F Saurini, S Salam, C Joubert, M Hary-Lenglet, A Lecomte, D-Y Yefsah, P Venault, S Berrard, M Nosten-Bertrand, Y Clément, G Vodjdani

Chronic stress represents a major environmental risk factor for mood disorders in vulnerable individuals and dysfunctional monoamine systems have been associated with anxiety and depression. In the mouse, unpredictable chronic mild stress (UCMS) mimics the chronic socio-environmental stress, which may lead to anxio-depressive episodes in humans. Here, we investigated the consequences of a serotonin (5-HT) deficit in neurons of the dorsal raphe nucleus (DRN) on emotional behaviors before and in response to UCMS. We have first validated a UCMS protocol in adult male C57BL/6 mice to model the anxio-depression-like syndrome. This protocol induced strong behavioral changes as assessed by a battery of tests measuring anxiety and depression-like behaviors. It also modified the expression of genes encoding molecules of the neurotransmitter systems. We next investigated the impact of selectively blocking serotonergic neurotransmission. This was achieved by inhibiting 5-HT synthesis in a sub-region of the brainstem, the DRN. We have selected and tested for efficiency an interfering RNA against tryptophan hydroxylase 2 (Tph2) mRNA. A lentiviral vector co-expressing the corresponding shRNA and the GFP reporter gene was injected stereotactically into the DRN of adult mice. Following UCMS and behavioral assessment, efficiency of TPH2 inhibition in the brainstem was determined by immunohistochemistry. In brain regions of serotonergic projections, reduction of 5-HT synthesis was determined by HPLC dosage and expression of genes of neurotransmission systems was analyzed by q-RT-PCR. Such studies should contribute to establish functional maps of serotonergic nuclei and clarify the links between neurotransmission impairment, anatomical and biochemical changes and functional deficits.

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A continuous fully automated progressive ratio task for the IntelliCage

AK Fritz, DP Wolfer
Progressive ratio tasks are well established to study the rewarding impact of drugs of abuse and food in rodents. However, their current implementation in operant chambers is complex and tedious. Animals are tested individually during many sessions and often need to be food deprived.

We have implemented a progressive ratio task for mice in the IntelliCage where animals are group-housed and can be tested 24/7 without deprivation and human interference. Each learning corner offers a choice between water and saccharin solution. Water access always requires a single nosepoke. The number of pokes needed to access the saccharin solution increases each time the mouse chooses saccharin but decreases after each choice of water.

C57BL/6 mice reached a stable equilibrium within few days, making on average 5-10 nosepokes to access saccharin. An equilibrium was also established rapidly in an aversively motivated version of the task, with mice making similar numbers of nosepokes to avoid drinking quinine. Modification of the available choices rapidly led to an adapted equilibrium reflecting the new value difference between stimuli.

This new IntelliCage task assesses the motivation of mice to work for reward or to avoid aversive stimuli. It is efficient, requires no handling, isolation or deprivation. Because an equilibrium is established, motivation can be monitored continuously over extended periods of time.

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58.

Autosomal-dominant sensory ataxia linked to the loss of RNF170 function
Autosomal-dominant sensory ataxia (ADSA) is a rare hereditary motor disorder, which predominantly entails a progressive gait abnormality. Previous studies have reported that the ADSA is linked to a missense mutation (595C>T) in the RING finger protein 170 (RNF170) gene, which encodes an E3 ubiquitin ligase to mediate degradation of type-I inositol 1,4,5-trisphosphate receptors (ITPR1). In spite of these findings, the functional identity thereof has remained unclear. Herein, we generated mice lacking RNF170 (RNF170−/−) to evaluate the effect on the loss of RNF170, and found that RNF170−/− mice exhibited age-dependent gait abnormality at 12-month-old. While wild-type littermates showed stable gait performances with synchronous diagonal limb movements, RNF170−/− mice showed unstable walking with disputed inter-limb coupling with a fixed step sequence of four paws. Remarkably, as reported in ADSA patients, RNF170−/− mice showed a reduced sensitivity for proprioception and thermal nociception. We also found that the amount of ITPR1 protein, which eliminated through the RNF170 function, was elevated in the spinal cord and cerebellum of RNF170−/− mice, but no significant changes in the cerebral cortex. Collectively, these results suggest that ADSA is associated with the loss of RNF170 function, and our mouse model will contribute to reveal the physiological mechanisms underlying coordinated limb movements.

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