Genes, Brain and Behaviour 2010

12th Annual Meeting of the International
Behavioural and Neural Genetics Society

May 12 – 16, 2010
Halifax, Nova Scotia, Canada

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Dalhousie University Faculty of Science
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Wiley-Blackwell Publishers (Genes, Brain and Behavior)
Oxford Gene Technology

PROGRAM

Wednesday 12 May

14:00-16:00 **Workshop 1: Allen Brain Atlas**
Amy Bernard
McCain Building, Scotiabank Auditorium

16:00-16:30 Coffee Break

16:30-18:30 **Workshop 2: JAX Phenome Database**
Terry P. Maddatu
McCain Building, Scotiabank Auditorium

18:30-19:30 **Public Lecture: Was Darwin Wrong (About the Tree of Life)?**
W. Ford Doolittle
McCain Building, Scotiabank Auditorium

19:30-21:00 **Welcome Reception**
Dalhousie University Faculty Club

Thursday 13 May

9:00-10:00 **Plenary Lecture 1**
Mary-Anne Enoch
A multi-directional approach to the detection of genetic risk for alcohol dependence
McCain Building, Scotiabank Auditorium

10:00-10:30 Coffee Break

10:30-12:30 **Symposium 1: Physical activity and drug abuse**
Organizers: MA Ehringer & J Rhodes
McCain Building, Scotiabank Auditorium

Angela Ozburn
Wheel running, voluntary ethanol consumption, and hedonic substitution

Marissa Ehringer
The effects of voluntary wheel-running on alcohol consumption: Common responses across different genetic strains of mice

Alan Rosenwasser
Wheel-running: A novel phenotypic marker for affective disruption during ethanol withdrawal?

Justin Rhodes
Wheel running exercise delays extinction of conditioned place preference for cocaine in male C57BL/6J mice in association with impaired exercise-induced adult hippocampal neurogenesis

12:30-13:30 Lunch

13:30-15:30 **Symposium 2: Behaviour genetics analysis in the collaborative cross**
Organized by EJ Chesler & LM Tarantino
McCain Building, Scotiabank Auditorium

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Behavioral genetic analysis in the collaborative cross and related populations

Abraham Palmer
Genetic dissection of anxiety-like behaviors using emergent CC mice

Justin Rhodes
Selective breeding for increased home cage physical activity in Collaborative Cross and Hsd:ICR mice

Robert Hitzemann
Gene networks in a Heterogenous Stock (HS) population of the Collaborative Cross (CC)

Lisa Tarantino
An interdisciplinary program for systems genomics of complex behaviors

15:30-16:00 Coffee Break

16:00-18:00 **Outstanding Young Investigator Talks**
McCain Building, Scotiabank Auditorium

Laurence Coutellier
TIP39 signaling as a modulator of the effects of emotional arousal on memory performance

William Giardino
Genetic dissection of the corticotropin-releasing factor (CRF) system reveals a role for the CRF type-2 receptor in locomotor sensitivity to methamphetamine

Viara Mileva-Seitz
Maternal genotype moderates the relationship between recent stress and maternal behavior

Megan Mulligan
Functional insertion of B2 SINE in Comt controls 3' UTR length and generates widespread expression and behavioral differences among inbred strains of mice

Friday 14 May

9:00-10:00 **Plenary Lecture 2**
Ann-Shyn Chiang
Neural circuitry governing CO_2 avoidance behaviour in the *Drosophila* brain
McCain Building, Scotiabank Auditorium

10:00-10:30 Coffee Break

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Organized by VJ Bolivar & JN Crawley
McCain Building, Scotiabank Auditorium

Jacqueline N. Crawley
Autism-like behavioral phenotypes in BTBR T+tf/J mice

Peter Nguyen
Modified synaptic plasticity in mice with reduced hippocampal commissures

Douglas Wahlsten
Crosses among four mouse strains afflicted with absent corpus callosum: Searching for major gene effects and behavioral correlates

Valerie J. Bolivar
Genetic investigations of neuroanatomical and social behavior abnormalities in BTBR T+tf/J mice

12:30-13:30 Lunch
IBANGS Executive Meeting
McCain Building, Room #2016

13:30-14:30 **IBANGS General Business Meeting**
Everyone is encouraged to attend
McCain Building, Room #2016

14:30-15:30 **Open Talks 1: Stress, Anxiety & Psychiatric Disorders**
McCain Building, Scotiabank Auditorium

Y. Osee Sanogo
The threespine stickleback, *Gasteroteus aculeatus*, as a model for understanding the brain transcriptomic response to stress

Leanne Fraser
The triple test: Measuring anxiety- and locomotion-related behaviours in mice

Tsuyoshi Miyakawa
Immature dentate gyrus as a potential endophenotype for psychiatric disorders

15:30-19:00 **Poster Session**
Dalhousie University Faculty Club, 3rd Floor

Saturday 15 May

9:00-11:00 **Symposium 4: Selected works from Genes, Brain and Behavior**
Organized by J Dubnau
McCain Building, Scotiabank Auditorium

Kyung-An Han
Dopamine in behavioral disinhibition

Jerry Yin
Sleep-associated dCREB2-responsive transcription

Su Guo
Deciphering genetic and cellular networks controlling innate behavior in zebrafish

Josh Dubnau
Memories of a fly: Psychology, circuits, and genes

11:00-11:30 Coffee Break

11:30-13:30 **IBANGS Distinguished Scientist Award Lecture**
Tamara Phillips
Cool! I can breed for this!
McCain Building, Scotiabank Auditorium

Afternoon Free

Sunday 16 May

9:00-11:00 **Symposium 5: Genetically-based audiogenic seizure models**
Organized by CL Faingold & Il Poletaeva
McCain Building, Scotiabank Auditorium

Carl Faingold
Neuronal network for audiogenic seizures in the Genetically Epilepsy-Prone Rat (GEPR-9) – molecular mechanisms of network expansion

Inga Poletaeva
Krushinsky-Molodkina (KM) inbred rat strain: Audiogenic epilepsy, catalepsy, inheritance

Noberto Garcia-Cairasco
Epilepsy and neuropsychiatric comorbidities: Lessons from the Wistar Audiogenic Rat (WAR) strain

Prosper N'Gouemo
Altered voltage-gated calcium channels and signaling and inherited seizure susceptibility in the Genetically Epilepsy-Prone Rat (GEPR-3)

11:00-11:30 Coffee Break

11:30-13:00 **Open Talks 2: Genes, Brain & Alcohol**
McCain Building, Scotiabank Auditorium
Igor Ponomarev
Transcriptional networks in brains of alcoholic and nonalcoholic individuals

Angela Ozburn
Clock∆19 mutants exhibit increased ethanol preference and consumption

Christopher Kliethermes
Food deprivation induces strain-specific behavioral responses to ethanol in Drosophila melanogaster

John Crabbe
Alcohol and tastant preference drinking in mice selectively bred for High Drinking in the Dark (HDID-1)

13:00-14:00 Lunch

14:00-15:00 **IBANGS Young Investigator Award Lecture**
Hiroki Ishiguro
Analysis of NrCAM related molecular pathway underlying addiction
McCain Building, Scotiabank Auditorium

15:00-15:30 Coffee Break

15:30-17:00 **Open Talks 3: Receptors, Gene Expression & Brain Mapping**
McCain Building, Scotiabank Auditorium

Emmanuel Onaivi
CNS effects of CB2 cannabinoid receptors

	Elena Jazin Regional differences in sexually dimorphic gene expression in adult mice brain Amy Eisener-Dorman Using comparative analysis and haplotype mapping approaches to identify quantitative trait loci in closely related strains Robert Williams A whole genome sequence for DBA/2J and its use in reverse complex trait analysis
17:00-19:00	Free time, travel to dinner at Radisson Suites Hotel
19:00-00:00	**Gala Dinner at Radisson Suites Hotel**

WORKSHOP & PUBLIC LECTURE
ABSTRACTS
Wednesday May 12, 2010

14:00-16:00 Workshop 1 ***Wednesday May 12***
Amy Bernard

Fueling Discovery with the Allen Brain Atlas: Tools and data for exploring gene expression in the brain
A. Bernard
Associate Director of R&D, Methods Development, Allen Institute for Brain Science, Seattle, WA

The Allen Institute for Brain Science has developed a suite of web-based tools and datasets, created with the hope that researchers will utilize these resources to drive multiple areas of scientific discovery in neuroscience. An overview of ideas and research pathways that have emerged from the analysis of this publically-accessible data will be presented, focusing on using gene expression as a means for classifying neuronal cell types and anatomical boundaries. This will be followed by a user training workshop that will demonstrate a subset of the online resources available through the Allen Brain Atlas web portal, for exploring gene expression in the mouse and human brain. Basic data access, navigation and usability will be covered, as well as more sophisticated search and visualization features.

The workshop will cover the full spectrum of Allen Institute resources available at http://www.brain-map.org/, including those for adult mouse brain, developing mouse brain, mouse spinal cord, human cortex, transgenic mice resources, and more.

16:30 -18:30 Workshop 2 ***Wednesday May 12***
Terry P. Maddatu

Mouse Phenome Database (MPD)
T.P. Maddatu, S.C. Grubb, C.J. Bult & M.A. Bogue
The Jackson Laboratory, Bar Harbor ME, USA

MPD (www.jax.org/phenome), maintained at The Jackson Laboratory, is the product of an international community effort to collect phenotypic and genotypic data on laboratory mouse strains. Since last year (2009), MPD has added several comprehensive datasets, including autism-relevant behaviours, toxicogenetic analysis of susceptibility to alcohol intoxication and drug-induced liver injury, aging-related phenotypes, preferences for different concentrations of sucrose, high-fat diet-induced effects, and reproductive, vestibular, adiposity, and eye parameters. MPD is also in the process of integrating quantitative data from gene expression datasets, which will be eligible for analysis with MPD tools.

MPD is indispensible for helping researchers select optimal strains for many research applications. MPD contains:
• Data for over 600 strains of mice
• Hundreds of baseline measurements of biomedically-relevant phenotypes
• A growing collection of data from treated mice, e.g., drugs, carcinogenic or toxic compounds
• Detailed protocols and environmental conditions of the test animals
• New SNP datasets (more strains and more genomic locations)
• Pathological survey of aged inbred strains of mice (pending)
• Gene expression microarray data enhancing data mining efforts (pending)

18:30-19:30 Public Lecture ***Wednesday May 12***
W. Ford Doolittle

Was Darwin Wrong (About the Tree of Life)?
W.F. Doolittle
Biochemistry & Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada
(w.ford.doolittle@dal.ca)

Molecular phylogeneticsts have devoted much of the last three decades to reconstructing a "universal Tree of Life" from gene sequences. But most of Life's history is prokaryotic, and for prokaryotes, gene transfer across species line (even between "phyla") is so frequent as to make any such reconstruction very difficult. Several of us have argued that in fact gene transfer renders the whole Tree concept meaningless, and a key part of Darwin's theory, as it was formulated by him and as it has been spun by neoDarwinists, wrong. Staunch defenders of evolution against creationism object to such interpretations, of course. They should not, because getting rid of the Tree would allow us to recast evolutionary theory in a much more defensible form. I see this episode in the history of my science as an object lesson which might be of use to other disciplines.

TALK ABSTRACTS

Thursday, May 13, 2010

9:00-10:00 Plenary Lecture ***Thursday May 13***
Mary-Anne Enoch

A multi-directional approach to the detection of genetic risk for alcohol dependence

M.-A. Enoch
Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892, USA

Chronic heavy alcohol consumption has widespread pathological effects in the human brain and may lead to addiction. This involves modulation of two interacting systems that are fundamental to positive and negative reinforcement: the mesolimbic dopamine “reward” pathway and the stress response system. Alcohol dependence, a common, heterogeneous psychiatric disorder with a lifetime prevalence of 12.5%, has a heritability of approximately 50%. Genetic vulnerability is likely to be due to variation in numerous genes with small to modest effects in many neurotransmitter systems and signal transduction pathways within the reward pathway and stress response systems. Furthermore, risk variants in stress-related genes may only be operative in individuals exposed to childhood trauma.
This talk will approach the challenge of dissecting genetic risk for alcohol dependence from several directions. Firstly, data will be presented from three high-throughput technologies for identifying candidate genes and the risk variants therein. An overview of a genome-wide assessment of the transcriptome in postmortem human hippocampus will be presented. Extensive genome-wide, mRNA changes in alcoholics and cocaine addicts within addiction-related coherent pathways will be discussed followed by analyses of up- and down-regulation of expression within stress-response gene clusters and pathways, such as GABAergic, that are highly pertinent to addiction vulnerability. Results from a dense whole genome linkage scan and a genome-wide association study will be presented, showing that the use of intermediate phenotypes (such as electrophysiological measures) for alcoholism is much more successful at detecting candidate genes and risk variants than the heterogeneous diagnostic phenotype. Secondly, results from case-control association studies within candidate gene clusters, notably GABAergic and serotonergic, and gene-environment interaction studies in stress-related genes will be presented. Finally, the overall findings will be discussed in the context of pharmacogenetics and potential drug targets.

10:30 Symposium 1: Talk ***Thursday May 13***
Angela Ozburn

Wheel running, voluntary ethanol consumption, and hedonic substitution

A.R. Ozburn[1], R.A. Harris[2] & Y.A. Blednov[2]
[1]University of Texas Southwestern Medical Center, Dallas, TX 75235 USA; [2]Waggoner Center for Alcohol and Addiction Research, Institute for Neuroscience, University of Texas at Austin, Austin, TX 78712, USA.

Enhanced environments, ethanol consumption and wheel running are reinforcing stimuli when presented individually to rodents (Nowak et al., 2000; Middaugh and Kelley, 1999; Samson et al., 2000; Werme et al., 2002). Several rodent models of ethanol self-administration have been developed to characterize specific aspects of alcohol drinking, such as initiation, binge drinking, maintenance/chronic, withdrawal, and craving/relapse (Lovinger and Crabbe, 2005). We are interested in investigating the behaviors of mice that have chronically consumed alcohol, as it has been shown that C57BL/6J mice display signs of ethanol dependence after three weeks of continuous drinking (Phillips et al., 1994). The primary goal of the present study was to investigate the relationship between wheel running and consumption of ethanol in ethanol preferring C57BL/6J mice. Wheel running has been shown to be rewarding and anti-depressive in several mouse strains (Brene et al., 2007). Wheel running activates brain reward pathways known to be responsive to drugs of abuse (Nestler, 2005; Vargas-Perez et al., 2003). Although natural and drug reinforcers activate similar brain circuitry, there is behavioral evidence suggesting they differ in perceived value. Hyman et al. (2006) suggest that the value of addictive drugs increases at the expense of natural rewards without serving to increase health. The primary goal of the present study was to investigate the relationships between the reinforcing behaviors of wheel running and ethanol consumption in C57BL/6J mice with a history of chronic ethanol consumption. Mouse behaviors were observed after the following environmental manipulations: standard or enhanced environment, accessible or inaccessible wheel, and presence or absence of ethanol. Using a high-resolution volumetric drinking monitor and wheel running monitor, we evaluated whether alternating access to wheel running modified ethanol-related behaviors and whether alternating access to ethanol modified wheel running or subsequent ethanol-related behaviors. We found that ethanol consumption remained stable with alternating periods of wheel running. However,

wheel running increased in the absence of ethanol and decreased upon reintroduction of ethanol. Upon reintroduction of ethanol, an alcohol deprivation effect was seen. The decreased wheel running and increased ethanol consumption could represent hedonic allostasis, a deviation in the natural reward and anti-reward processes in order to maintain a deceptive stability. Collectively, the results support theories of hedonic substitution and suggest that female C57BL/6J mice express ethanol craving under these specific conditions.

This research was supported by the Integrative Neuroscience Initiative on Alcoholism Consortium Grant AA13520, and NIH Grants AA06399-S and AA16424.

11:00 Sympsium 1: Talk ***Thursday May 13***
Marissa Ehringer

The effects of voluntary wheel-running on alcohol consumption: Common responses across different genetic strains of mice
M.A. Ehringer, N.R. Hoft & J. Godfrey
Institute for Behavioral Genetics, Department of Integrative Physiology, University of Colorado, Boulder, CO, 80309, USA

An emerging theme in addiction research is the hypothesis that there may be common molecular pathways which underlie different types of addiction (Nestler 2005; Volkow and Wise 2005). Previous work from our lab has shown that the high-drinking C57BL/6Ibg mice voluntarily consume less alcohol in a two-bottle choice paradigm when given access to a running wheel (Ehringer et al, 2009). Recently we have tested several other inbred strains of mice to determine whether this phenomenon can be generalized to other genetic backgrounds. Thus far, all strains we have tested (DBA/2Ibg, 129 Svev/Taconics, C3H/2Ibg, A/J, and high and low activity selected strains) show the same trend of decreased alcohol preference in the presence of a running wheel. In summary, we believe this combined alcohol preference and wheel running mouse model may be a good approach for future studies aimed at understanding which genes and neuronal pathways may be co-regulated by alcohol and exercise, thus providing greater insight into possible prevention and treatment approaches for human alcoholism. Supported by NIAAA AA015336 and AA017889.

11:30 Symposium 1: Talk ***Thursday May 13***
Alan Rosenwasser

Wheel-running: A novel phenotypic marker for affective disruption during ethanol withdrawal?
A.M. Rosenwasser & R.W. Logan
Department of Psychology, School of Biology and Ecology, and Graduate School of Biomedical Sciences, University of Maine, Orono, ME 04469

Alcohol withdrawal is associated with behavioral, affective, and neurophysiological disturbances in both human alcoholics and in animal models of alcohol dependence. These effects include disruptions in sleep and circadian rhythms as well as increased expression of anxiety- and depression-like behaviors. In order to expand the phenotypic characterization of ethanol withdrawal, we have been examining the effects of withdrawal from chronic intermittent exposure to ethanol vapor (CIE) vapor exposure on circadian patterns of running-wheel activity in inbred (C57BL/6J and C3H/HeJ) mice. Following baseline activity measurements, mice were exposed to either one, two or three 4-day CIE treatment cycles in which 16 hours of daily ethanol vapor exposure alternated with 8 hours of exposure to plain air, while control animals were exposed only to plain air in an identical environment. Ethanol exposure began at the onset of the dark phase of the daily 12:12 LD cycle, and each exposure period was initiated by an injection of 1.6 g/kg ethanol and 1.0 mmol pyrazole, i.p., to rapidly stabilize blood ethanol concentrations, while controls received pyrazole in saline only. In both strains, ethanol withdrawal was associated with pronounced reductions in wheel-running, but these effects were more profound and more enduring in the “withdrawal-sensitive” C3H/HeJ strain relative to the “withdrawal-resistant” C57BL/6J strain. Further, monitoring of home-cage water drinking via contact-sensing lickometers showed no effects of ethanol withdrawal, indicating that the observed reductions in locomotor activity are not due to non-specific factors such as malaise or lethargy. Instead, these effects probably reflect the specific biobehavioral significance of running-wheel activity, generally considered to be an intrinsically rewarding form of motivated exploratory behavior. Subsequent experiments will utilize a wider range of mouse strains in order to characterize the neurogenetic bases of withdrawal-related hypo-locomotion.
Supported by NIAAA AA013893 and by INIA-Stress.

12:00 Symposium 1: Talk ***Thursday May 13***
Justin Rhodes

Wheel running exercise delays extinction of conditioned place preference for cocaine in male C57BL/6J mice in association with impaired exercise-induced adult hippocampal neurogenesis
J. Rhodes
Department of Psychology, University of Illinois, Urbana, IL, 61801, United States

The interaction between aerobic exercise and drug abuse is relatively unexplored. It deserves attention because recent data suggest that neuroadaptations from exercise promote learning in circuits that overlap with drug abuse. The hippocampus is an important point of intersection because it is a major locus for change from aerobic exercise and it plays a central role in contextual conditioning. Specifically, contextual cues paired with drugs trigger emotional responses related to craving and relapse. Growing evidence suggests that exercise can enhance plasticity in the hippocampus in part by growing new nerve cells in the dentate gyrus. This could promote brain health and could potentially be useful in treatment of drug abuse. On the other hand, drug exposure is known to decrease neurogenesis and the outcome when combined with exercise is not known. Male C57BL/6J mice (n=80 total), 7 weeks of age, either received 10 place conditioning trials with 10 mg/kg cocaine, or were similarly exposed to the place conditioning apparatus but did not receive injections. After conditioning, the animals were either left in their standard cages or were placed into cages with a running wheel for 30 days. The first 10 days animals received daily injections of bromodeodyuridine (BrdU) to label dividing cells. On day 25, one group received a final day of conditioning with cocaine. On days 27-30 animals were tested for conditioned place preference, then euthanized to measure adult hippocampal neurogenesis by immunohistochemical detection of BrdU and neuronal nuclear protein (NeuN). Running significantly delayed extinction of conditioned place preference for cocaine. Cocaine treated animals displayed similar levels of neurogenesis as compared to untreated animals in the sedentary condition but significantly reduced adult hippocampal neurogenesis in the runner condition. Results suggest that exercise can delay extinction of cocaine conditioned place preference and that this behavioral rigidity is associated with significantly reduced exercise-induced adult hippocampal neurogenesis in male C57BL/6J mice.

13:30 Symposium 2: Talk ***Thursday May 13***
Elissa Chesler

Behavioral Genetic Analysis in the Collaborative Cross and Related Populations
E.J. Chesler
Oak Ridge National Laboratory, Oak Ridge, TN & The Jackson Laboratory, Bar Harbor, ME, USA

The Collaborative Cross is a systematic cross of eight inbred strains designed by members of the Complex Trait Consortium to create a large and genetically diverse panel of recombinant inbred strains. From five common and three wild-derived lines, systematic crosses result in a population with high power, precision and genetic diversity. This population and its derivatives are an exciting new resource for neurobehavioral genetic s. Several projects using the Collaborative Cross mice or related populations are in progress and highlight the utility of this resource. Inbreeding of the lines results in a genetic reference population, used for powerful mapping and genetic correlation analysis. Outcrossing of the lines to make a heterogeneous stock population yields a highly-precise, diverse population for mapping and selection experiments. Several hundred lines were established in the largest US effort to produce the Collaborative Cross, which was subject to genetic and behavioral characterization. A subset of lines from this population has been delivered for production of recombinant inbred (UNC) and Diversity Outcross (JAX) populations. Dr. Chesler will introduce the populations and the speakers.
Funded by: Office of Biological and Experimental Research, Office of Science, US Department of Energy; The Ellison Medical Foundation; NIH CA134240; University of Tennessee.

13:55 Symposium 2: Talk ***Thursday May 13***
Abraham Palmer

Genetic dissection of anxiety-like behaviors using emergent CC mice

A.A. Palmer[1,2], G. Sokoloff[2], V.M. Philip[3], M. Beckmann[4], G.A. Churchill[5] & E.J. Chesler[4,5]
[1]Department of Human Genetics, University of Chicago; [2]Department of Psychiatry and Behavioral Neuroscience, University of Chicago; [3]Genome Science and Technology Program, University of Tennessee, Knoxville, TN; [4]Oak Ridge National Labs, Oak Ridge, TN; [5]The Jackson Laboratory, Bar Harbor, ME.

Understanding the genetic factors that influence individual variability in fear learning and anxiety-like behavior has been a long standing goal of behavioral geneticists. We have completed a multi-center collaborative project to evaluate several anxiety-like behaviors in mice from G2:F5 through G2:F7 generations of the emergent CC at Oak Ridge National Labs. The results of the analysis of more than 400 individuals from more than 200 funnels identify multiple genome-wide significant as well as suggestive QTLs for traits related to the open field and the light/dark box tests. These QTLs identify some of the same regions identified in more traditional studies, but with greater precision. Nevertheless, these regions still contain multiple genes and will require follow-up studies to identify the specific genes. In this regard we have made progress using an even more highly recombinant advanced intercross line (AIL), which offers further improvements in mapping precision. Methods developed for the analysis of AILs will also be valuable for the analysis of outbred populations made up of the strains used to create the collaborative cross. The integration of these tools should bring to fruition the decades-long quest to identify the genes responsible for variability in anxiety-like behavior among mammalian model organisms.
Funded by: MH079103

14:20 Symposium 2: Talk ***Thursday May 13***
Justin Rhodes

Selective breeding for increased home cage physical activity in Collaborative Cross and Hsd:ICR mice
J. Rhodes, J. Zombeck & E. DeYoung
Department of Psychology, University of Illinois, Urbana, IL, 61801, United States

One powerful method for discovering how genes influence behavior is to conduct a long-term, replicated, selective breeding experiment. Physical activity is of broad interest for biomedicine because variation in physical activity is implicated in obesity, depression, ADHD, cognitive aging among other disorders. Locomotor activity in the open field and wheel running behavior have been the focus of previous selective breeding experiments using mice, but to our knowledge, distance traveled over several days in the home cage has never been used as the selection criterion. Therefore, we used TopScan video tracking software to measure home cage distance continuously over 6 days. We used modified cages with clear plastic tops and food and water delivered from the side. Here, I will report results of 5 generations of within-family selection starting from two separate populations of mice, G2:F1 Collaborative Cross (CC) and Hsd:ICR. In addition, wheel running and open field behavior were measured in generations 0 and 1 to estimate heritability and genetic correlations with home cage activity. In CC, all three activity traits showed significant heritability and moderate genetic correlations and a significant response to selection was observed for home cage activity. In Hsd:ICR strain, only wheel running was significantly heritable and we observed no response to selection on home cage activity. We propose to develop a long-term replicated selective breeding experiment for home cage physical activity using CC as the founding population. The rationale is to discover the origin of variation in voluntary physical activity at multiple levels of biological organization from genes to physiology to behavior.

14:45 Symposium 2: Talk *Thursday May 13*
Robert Hitzemann

Gene networks in a Heterogeneous Stock (HS) population of the Collaborative Cross (CC)

R. Hitzemann, O. Iancu, P. Darakjian, N. Walter, B. Malmanger, D. Oberbeck, J. Belknap & S. McWeeney
Departments of Behavioral Neuroscience, Medical Informatics & Clinical Epidemiology and Public Health & Preventative Medicine, Oregon Health & Science University, Portland OR 97239 and the Research Service, Veterans Administration Medical Center, Portland, OR 97239

In 2005, we formed 32 unique crosses from the 8 CC progenitor strains. Rather than inbreeding the animals, they were outbred using a circle breeding design. At G7 the number of families was increased to 48 by breeding a male from family 1 with a female from family 2 and so on. In the current study we have compared striatal gene expression (Illumina WG 6.1 arrays) in the HS-CC (G12), in the HS4 (G19) formed by crossing the C57BL/6J (B6), the DBA/2J (D2), the BALB/cJ and LP/J strains and in a B6xD2 F2. Sample sizes were 96, 56 and 58, respectively. The two strips on the WG 6.1 arrays were normalized separately and probes were masked using the SNP data available at http://www.sanger.ac.uk/modelorgs/mousegenomes/. The resulting expression data were used to determine if the genes (transcripts) expressed and their associated co-expression profiles were similar in the HS-CC, HS4 and F2 populations, despite the marked differences in genetic diversity (Roberts et al. 2007). There were no substantial differences among the three samples in the transcripts detected as being expressed and the variance of the expressed transcripts. Co-expression networks (modules) were constructed for the three samples as described by Zhang and Horvath (2005). The power-transformed Pearson correlation coefficients between gene pairs were used to infer a measure of connection strength or topological overlap; a hierarchical clustering procedure was used to identify distinct modules. In general, the modules detected in the F2 and HS4 aligned well with the modules detected in the HS-CC. We also examined the expression data using a "seeded" network procedure for genes of behavioral interest. Here we simply note the results for Drd2. The range of Drd2 expression was similar in the F2, HS4 and HS-CC populations (251, 224 and 273 percent, respectively). The Drd2 expression networks ($r > 0.56$, $p \sim < 10\text{-}5$) were similar in size ($N = \sim 1000$) and composition e.g. Pde2a, Pde10a, Rgs4, PPP1r1b and Actn2 were associated with Drd2 expression at $r < 0.75$ in all three populations. Overall, the data

suggest that in the context of the parameters described above, the HS-CC is more similar than different from the F2 and HS4. The question of whether or not the regulation of expression is also similar is currently under investigation.

Supported by MH51372, AA 13484, AA 11034, AA 010760 and VA Research.

15:05 Symposium 2: Talk ***Thursday May 13***
Lisa Tarantino

An interdisciplinary program for systems genomics of complex behaviors
L.M. Tarantino[1], S.S. Moy[1,2], D. Threadgill[3,4], G. Churchill[5], P. Sullivan[1,4] & F. Pardo-Manuel de Villena[4]
[1]Department of Psychiatry, University of North Carolina, Chapel Hill, NC 27599; [2]Neurodevelopment Research Center, University of North Carolina, Chapel Hill, NC 27599; [3]Department of Genetics, North Carolina State University, Raleigh, NC; [4]Department of Genetics, University of North Carolina, Chapel Hill, NC 27599; [5]The Jackson Laboratory, Bar Harbor, ME

Psychiatric disorders are among the least tractable for human genetic studies. Genomewide association studies (GWAS) for psychiatric disorders are starting to yield interesting results but difficulties obtaining adequate sample sizes and at the basic level of disease diagnosis persist. Animal models have been used extensively to study psychiatric disorders. These efforts have generally been aimed at analysis of endophenotypes - underlying components of the disease with simpler genetic architecture that may be more amenable to genetic analysis. Until now, much of this work in mice has been accomplished with inbred strain crosses. However, observation of behavior in individual strains and two-way crosses limits the amount of genetic variation available for analysis. More recently, a new mouse resource, the Collaborative Cross (CC), has been generated. This eight-way cross between diverse inbred strains increases both genetic and phenotypic diversity resulting in a mouse population that more accurately mimics the genetic diversity observed in humans. Genetic diversity can be further exploited by producing recombinant intercross animals from the inbred CC strains (CC-RIX). We recently initiated a large-scale behavioral, genetic and genomic analysis of CC-RIX mice that includes assessment of anxiety, depression, stress and social interaction behaviors. As an important component of the study, we are assessing gene by environment interactions by exposing the mice to three different early environments at weaning - isolate housing, standard group housing and enriched housing. Preliminary results from the CC parental strains and reciprocal F1 mice indicate that early housing environment has a marked effect on anxiety-related behaviors and that mouse strains respond differently to the home cage environment during postnatal development.
NIH/NIMH P50-MH090338

16:00 Talk ***Thursday May 13***
Laurence Coutellier

TIP39 signaling as a modulator of the effects of emotional arousal on memory performance
L. Coutellier, A. Logemann & T. Usdin
Section on Fundamental Neuroscience, NIMH, NIH, Bethesda, MD, USA, 20892

Tuberoinfundibular peptide of 39 residues (TIP39) is a neuropeptide localized to neural circuits subserving emotional processing. Recent studies showed that mice with null mutation for the gene coding TIP39 (TIP39 KO mice) display increased susceptibility to environmental provocation. Based on this stressor-dependent phenotype, the neuroanatomical distribution of TIP39, and knowledge that arousing conditions alter memory functions via noradrenergic activation, we hypothesized that emotional arousal from novel environment exposure differently affects memory performance of mice with or without TIP39 signaling, potentially by differences in sensitivity of the noradrenergic system. We tested TIP39 KO mice and mice with null mutation of its receptor, the parathyroid hormone 2 receptor (PTH2-R), in three memory tasks under conditions of high or low emotional arousal. Under high arousal, but not under low arousal conditions, mice lacking TIP39 signaling demonstrated consistent memory impairment. We observed similar findings in wild-type mice infused i.c.v. with a PTH2-R antagonist prior to testing. Furthermore, TIP39 KO mice had increased c-fos activation in the locus coeruleus under high arousal suggesting a role of the noradrenergic system. This idea was further supported by the complete restoration of memory functions after injection of a beta-adrenoreceptor-blocker. Collectively, these results suggest that TIP39 signaling modulates the effects of arousal on memory performance, probably by acting on noradrenergic signaling.

16:30 Talk ***Thursday May 13***
William Giardino

Genetic dissection of the corticotropin-releasing factor (CRF) system reveals a role for the CRF type-2 receptor in locomotor sensitivity to methamphetamine

W.J. Giardino, A.M.J. Anacker, E. Spangler, J. Li, D.M. Cote, T.J. Phillips, & A.E. Ryabinin
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Sensitivity to the locomotor-activating properties of commonly-abused drugs, such as ethanol (EtOH) and methamphetamine (MA), may contribute to risk for dependence. The corticotropin-releasing factor (CRF) system, comprised of four endogenous peptides (CRF, Urocortins 1, 2, and 3), and two receptor subtypes (CRF-R1, CRF-R2), has been implicated in various endophenotypes associated with substance abuse, including sensitivity to drug-induced locomotor activity. The CRF system exhibits complex pharmacological relationships (CRF and Urocortin 1 bind to both receptors, while Urocortin 2 and Urocortin 3 are selective for CRF-R2) and distinct, yet partially overlapping, patterns of brain expression.

Previous work examining the contribution of the CRF system to EtOH-induced behavior has identified a role for CRF and CRF-R1, showing that activation of the hypothalamic-pituitary-adrenal (HPA) axis by CRF-R1 is requisite for EtOH-induced locomotor sensitization. In order to assess whether the complement of CRF system components involved in sensitivity to MA is similar to that involved in sensitivity to EtOH, we compared the locomotor response to MA in four lines of genetic knock-out mice, each deficient for a single component of the CRF system (CRF-R1, CRF-R2, CRF, or Urocortin 1).

In this protocol, horizontal locomotor activity was measured for 15 minutes immediately following an intraperitoneal injection of saline (Days 1, 2, and 12) or 1 mg/kg MA (Days 3, 5, 7, 9, 11 and 27). This protocol allows us to derive a number of variables from the data, including the acute stimulant response to MA (Day 3 initial MA activity score - Day 2 saline baseline activity score), the magnitude of repeated MA-induced sensitization (Day 11 MA activity score - Day 3 initial MA activity score), and the long-term expression of sensitization to MA following a two-week period of abstinence (Day 27 final MA activity score - Day 11 MA activity score).

Our results demonstrate that, unlike EtOH, MA-induced locomotor activation is attenuated by the deletion of CRF-R2, but not CRF-R1, as CRF-R2 KO mice showed significantly decreased acute MA-induced stimulation. Furthermore, because CRF KO mice showed no difference in acute MA-induced stimulation (and showed enhanced, rather than diminished, repeated MA-induced sensitization), this suggests that urocortin peptides, rather than CRF, are likely to underlie CRF-R2-dependent acute stimulation to MA. Results from immunohistochemical mapping techniques, in which the immediate early gene c-Fos was used as a marker of neural activation, indicate that the neural circuitry underlying CRF-R2-dependent locomotor sensitivity to MA likely involves the central and basolateral nuclei of the amygdala. Future studies are focused on further elucidating the specific CRF-R2-specific neural circuitry involved in locomotor sensitivity to MA and other behavioral traits relevant to MA addiction.
Supported by NIDA: P50DA018165, T32DA007262

17:00 Talk ***Thursday May 13***
Viara Mileva-Seitz

Maternal genotype moderates the relationship between recent stress and maternal behavior

V. Mileva-Seitz, J. Kennedy, R. Levitan, L. Atkinson, M. Steiner, M. Sokolowski & A. Fleming
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Human maternal behavior is a set of maternal responses to infant needs and cues, and is influenced by many factors. We recently found that maternal genotype may moderate the relationship between early life adversity and maternal behavior, and we wanted to examine if it similarly moderates the relationship between recent stressors and maternal behavior. DNA from 200 mothers was genotypes for markers in two candidate gene families - dopamine (DA) and serotonin (5HT). At 6 months postpartum, we obtained scores from the mothers for five categories of recent stress: “marital strain”, “marriage quality”, “acute stress”, “chronic stress”, and “perceived stress”; and computed durations for maternal behaviours (e.g. mother “touching” and “vocalizing” to infant) during a 30 minute recorded mother-infant interaction. In the 5HT system, we found a significant interaction effect between genotype at a 5HT transporter promoter polymorphic region (5HTTLPR) and “marriage quality” on “touching” ($F(1, 104) = 12.8$; $p = .001$). In the DA system, a polymorphism on the DA receptor 4 (DRD4 Exon III VNTR) significantly interacted with “marriage quality” as a predictor of “vocalization” ($F (1, 109) = 9.2$; $p = .003$). The other stress variables showed less significant interactive effects with genotype. Interestingly, DRD4 VNTR and 5HTTLPR genotypes interacted to predict “perceived stress” ($F (1, 112) = 10.2$; $p = .002$). We conclude that 1) how mothers rate their “marriage quality” is strongly moderated by both DA and 5HT genotypes to influence maternal behaviors at 6 months postpartum; and that some aspects of recent stress are more predicted by genotype than others.

17:30 Talk ***Thursday May 13***

Megan Mulligan

Functional insertion of a B2 SINE in Comt controls 3' UTR length and generates widespread expression and behavioral differences among inbred strains of mice

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Catechol-O-methyltransferase (COMT) is a key enzyme responsible for the degradation of catecholamine neurotransmitters including dopamine and norepinephrine. Variation in COMT activity influences prefrontal cortical function, aggression, and drug response. In this study we report the identification and characterization of a novel murine sequence variant that regulates Comt mRNA, protein expression, and activity. We examined Comt mRNA levels in multiple tissues in over 90 genetically diverse strains and demonstrate consistent cis-regulation in recombinant inbred (RI) lines, including the C57BL/6J x DBA/2J BXD set. The only sequence difference between C57BL/6J (B6) and DBA/2J (D2) is a 230 bp insertion of a B2 family short interspersed element (B2 SINE) into the proximal 3' UTR of Comt in B6. This insertion introduces a premature polyadenylation signal and creates a short 3' UTR isoform. The B2 SINE is shared by a small subset of other strains, including A/J, BALB/cBy, AKR/J and LG/J, but is absent in other strains and wild subspecies of Mus. The short isoform is associated with increased protein expression in prefrontal cortex and hippocampus relative to the longer ancestral isoform. Comt genotype is also linked to expression differences of numerous genes involved in synaptic function, and modulates dopamine D1 and D2 receptor density in several brain regions.

Friday, May 14, 2010

09:00-10:00 Plenary Lecture ***Friday May 14***
Ann-Shyn Chiang

Neural Circuitry Governing CO_2 Avoidance Behavior In The *Drosophila* Brain
H.-H. Lin[1,3], L.-A. Chu[1,3] & A.-S. Chiang[1,2]
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Adult *Drosophila* can sense and avoid carbon dioxide (CO_2) emitted from neighboring stressed individuals. A single population of specialized olfactory sensory neurons has been shown to detect and relay CO_2 signal to the V-glomerulus in the antennal lobes. Here, we report a comprehensive map of neural circuitry connecting the V-glomerulus to higher brain centers. While the discovered V-glomerulus projection neurons were all sensitive to CO2 exposure, we found that the activation of two ventral-posterior-lateral (VPL) paired neurons is necessary and sufficient to elicit the CO_2–induced olfactory avoidance behavior.

10:30 Symposium 3: Talk ***Friday May 14***
Jacqueline N. Crawley

Autism-like behavioral phenotypes in BTBR T+tf/J mice
F. Roullet[1], M.L. Scattoni[1,2], J. Silverman[1], M. Wöhr[1,3], M. Yang[1] & J.N. Crawley[1]
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Autism is a neurodevelopmental disorder diagnosed by three categories of behavioral criteria: 1) abnormal reciprocal social interactions, 2) communication deficits, 3) stereotyped, repetitive behaviors with restricted interests. While the causes of autism remain unknown, the strongest evidence is genetic. Using a forward genetics strategy, we assayed social behaviors in 20 inbred strains of mice from the top tiers of the JAX Mouse Phenome Project. Robust social deficits were detected in BTBR T+tf/J (BTBR), corroborating initial findings by Valerie Bolivar.

BTBR is a little-known inbred strain that displays normal phenotypes on control measures of general health, developmental milestones, neurological reflexes, anxiety-related behaviors, learning and memory, sensory abilities, and motor functions. Our laboratory has been testing BTBR across a range of behavioral assays relevant to the three diagnostic symptoms of autism. 1) BTBR displayed an absence of sociability on our automated three-chambered social approach task. BTBR engaged in less reciprocal social interactions as juveniles and adults, as compared to highly social strains such as C57BL/6J (B6), FVB/NJ, and FVB/antJ. 2) As compared to B6, BTBR deposited fewer scent marks near a spot of fresh urine from an estrus B6 female. BTBR emitted fewer ultrasonic vocalizations (USV) than B6 in the presence of fresh urine from estrus B6 and BTBR females. BTBR pups separated from their dam and nest emitted more and louder USV than separated B6 pups. Adult BTBR emitted fewer USV than B6 in social situations as compared to B6 and FVB/NJ, particularly the frequency step category of calls. High levels of repetitive self-grooming were apparent in BTBR, at juvenile and adult ages, when tested in either the light or dark phases of their circadian cycle, when alone or with a partner, in both the home cage and novel environments.

No model system can be expected to fully recapitulate all endophenotypes of a human neuropsychiatric disorder. For example,

both male and female BTBR display social deficits, as opposed to the 4:1 male:female ratio in autism. However, BTBR incorporates elements of reduced social interactions, low olfactory and vocalization responses, and high repetitive behaviors, analogous to components of all three diagnostic symptoms of autism. Replication of social deficits in BTBR across multiple cohorts and in multiple laboratories has provided confidence in the robustness of this mouse model of autism. BTBR offers a translational tool to investigate genetic and biological mechanisms underlying low sociability, impaired communication, and high repetitive behaviors, and to discover therapeutics for the core features of autism.

Supported by the National Institute of Mental Health Intramural Research Program

11:00 Symposium 3: Talk ***Friday May 14***
Peter Nguyen

Modified synaptic plasticity in mice with reduced hippocampal commissures

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The hippocampus is critical for memory formation, and synaptic plasticity in hippocampal circuits contributes importantly to memory storage. Hippocampal commissures (HC) have been shown to be important for some aspects of memory extinction (Schimanski et al., J. Neurosci 22: 8277) and contextual fear conditioning (MacPherson et al., Brain Res. 1210: 179), but the contributions of the HC towards synaptic plasticity are unclear. BTBR mice lack intact HC and display agenesis of the corpus callosum; they are thus appropriate experimental subjects for probing the roles of these interhemispheric connections in synaptic plasticity. I present data showing that hippocampal slices from BTBR mice displayed intact expression of long-term potentiation (LTP), paired-pulse facilitation, and basal synaptic transmission in area CA1, compared to C57BL/6 (B6) mice with intact HC and corpus callosum. However, BTBR slices showed increased susceptibility to depotentiation (DPT), an activity-induced reversal of LTP. Because BTBR mice display reduction of the HC and agenesis of the corpus callosum, we conclude that these interhemispheric connections are critical for synaptic resistance to DPT. Resistance to DPT may contribute to consolidation of information storage in neural circuits (Woo and Nguyen, J. Neurosci. 23: 1125), and as such, enhanced DPT may be linked to some of the deficits in contextual fear memory previously observed in BTBR mice (Brain Res. 1210: 179). Further research is required to test more directly the roles of the HC per se, in the absence of corpus callosal abnormalities, in mediating this enhancement of depotentiation. In a broader perspective, these experiments reinforce the notion that studies of synaptic plasticity are essential for shedding light on the cellular mechanisms of behavioral phenotypes observed in diverse strains of inbred mice.

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11:30 Symposium 3: Talk ***Friday May 14***
Douglas Wahlsten

Crosses among four mouse strains afflicted with absent corpus callosum: searching for major gene effects and behavioral correlates

D. Wahlsten & M.O. Bohlen
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The inbred strains 129S1/SvImJ, BALB/cJ, BTBR T/+ tf J and I/LnJ show absence of the corpus callosum to varying degrees. Complementation tests done by intercrossing all of these strains provide useful information about the genetic architecture of the trait and help to plan an efficient search for major gene influences. Three-way and four-way crosses of the strains provide a means to dissect a system with a small number of genes having major effects. They also provide excellent material for assessing phenotypic behavioral correlates of absent corpus callosum, because the same phenotype can be generated for different genetic reasons. If a behavioral correlate appears in all types of genetic crosses, it is most likely a result of absent corpus callosum itself, rather than genetic pleiotropy. Data will be presented on motor coordination and social behavior in these complex crosses.

12:00 Symposium 3: Talk ***Friday May 14***
Valerie J. Bolivar

Genetic investigations of neuroanatomical and social behavior abnormalities in BTBR T+ tf/J mice
V.J. Bolivar[1,2], R.C. Auerbach[1,2] & G.W.M. Bothe[3]
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BTBR T+ tf/J (BTBR) mice display a virtual absence of the corpus callosum (CC), severe hippocampal commissure (HC) reduction and abnormal social behavior. As abnormalities in reciprocal social interactions are one of the hallmark characteristics of autism spectrum disorders, this inbred mouse strain may play an important role in elucidating some of the genes underlying this complex group of disorders. In our laboratory we are currently examining the role of genetics in the social behavior abnormalities seen in BTBR mice. To study social behavior we use the sociability assay developed by Dr. Jacqueline Crawley and colleagues, an "approach" assay that confines the initiation of social interaction to one mouse of a pair, thereby determining the level of sociability in a single animal. To examine the CC and HC abnormalities, we stained midsagittal sections using 0.2% gold chloride and measured commissure area using ImageJ software. In our genetic studies, we crossed BTBR to FVB/NJ, an inbred strain displaying fully formed commissures and a high level of social behavior. First, we crossed BTBR and FVB to produce reciprocal F1 hybrids [(BTBRxFVB)F1 and FVBxBTBR)F1]. We found that adult male F1 hybrid offspring from BTBR mothers were significantly less social than those from FVB mothers. Ongoing studies will determine if this difference is due to genetic and/or environmental factors. CC and HC areas were normal in both F1 populations. Next, using 140 individuals from a F2 population we performed a quantitative trait loci study with a 1449 single nucleotide polymorphism (SNP) panel (Medium Density Linkage Panel, Illumina, San Diego, CA) to discern chromosomal loci involved in the phenotypes of interest. Continuous ranges of social behavior and commissure areas were obtained in the F2 generation. Although we did not find any significant QTLs for social behavior, there was a suggestive locus on Chromosome 3. A highly significant locus on the distal end of Chromosome 4 was detected for midsagittal CC area, whereas a slightly more proximal region on Chromosome 4 was significantly linked with HC area. These loci do not overlap and thus may indicate that different genes are controlling the development of

these structures.
Funded by NIH and Taconic, Inc.

14:30 Open Talk ***Friday May 14***
Y. Osee Sanogo

The Threespine Stickleback, Gasteroteus aculeatus, as a Model for Understanding the Brain Transcriptomic Response to Stress
Y.O. Sanogo[1], S. Hankinson[1], D. Xie[3], M. Band[2], A. Obregon[1] & A.M. Bell[1]
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Predator related stress response in model organisms is being increasingly used as a new approach to understanding human emotional behavior and to investigating the plastic responses of the brain to acute or chronic stress. In this study, we used whole genome comparative oligonucleotide microarrays to investigate the brain transcriptomic response to predator cues using the threespine stickleback, Gasteroteus aculeatus as a model. Our study showed that exposure to olfactory, visual and tactile cues of predator (rainbow trout, Oncorhynchus mykiss) for six days resulted in subtle but significant transcriptomic changes in the brain of sticklebacks. Gene functional analysis and gene ontology (GO) enrichment revealed that the majority of the transcripts differentially expressed between the fish exposed to predator cues and the control group are primarily related to antigen processing and presentation (involving primarily the major histocompatibility complex MHC), transmission of synaptic signals, brain metabolic processes, gene regulation, or visual perception. Pathway analysis identified synaptic long-term depression, RAN signaling, relaxin signaling and phototransduction as the top four pathways that were over-represented. Our study shows that exposure of sticklebacks to predator cues results in the activation of a wide range of biological and molecular processes that can be identified using whole-genome expression analysis. We argue that the threespine stickleback is a robust model for understanding the ecological consequences of exposure to predator cues and for uncovering the molecular mechanisms of stress related disorders. Understanding the molecular and genetic responses to environmental stress has wide ranging implications for health, evolutionary biology and survival in extreme conditions.
Funded by: National Institutes of Health R01 GM082937 (AM Bell)

14:50 Open Talk ***Friday May 14***
Leanne Fraser

The Triple Test: Measuring anxiety- and locomotion-related behaviours in mice

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The most common procedure for investigating emotionality in mice is through the use of a battery of different behavioural tests. However, when using test batteries, the behaviour of the animal may vary between tests and one test may influence performance on subsequent tests. This study investigated, in mice, the validity of a new behavioural test of anxiety that consists of the physical integration of three simple and widely used tests of anxiety/emotionality: the open field (OF), elevated plus maze (EPM) and light/dark box (LDB) (Ramos, 2008, Trends in Pharmacol Sci 29: 493-498). Mice from four different strains (CD-1, BALB/cJ, DBA/2J, C57BL/6J) were used in a series of five experiments. Locomotor and anxiety-related behaviours were scored for all experiments in response to anxiety-modulating drugs. We found that CD-1 mice thoroughly explored the triple test, providing samples of behavioural measures from the three different tests throughout one testing session. Significant behavioural differences were found among three inbred strains (BALB/cJ, DBA/2J, C57BL/6J) and were not influenced by repeated testing. Both diazepam and alprazolam (4 and 2 mg/kg, respectively) increased the percentage of entries and reduced the amount of risk assessment towards the open arms of the EPM. The present findings show that the triple test is sensitive to genetic differences in anxiety- and locomotor-related behaviours. The results also suggest that the triple test, differently from individual models such as the EPM, might be adequate for longitudinal studies on genetic differences and drug effects. Finally, by providing quasi-simultaneous pictures of the behavioural state of a mouse in three different apparati, this new model adds complexity to the emotional phenotyping of mice while keeping it simple, rapid and inexpensive.
This research was supported by a grant from NSERC of Canada.

15:10 Open Talk ***Friday May 14***
Tsuyoshi Miyakawa

Immature dentate gyrus as a potential endophenotype for psychiatric disorders

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Elucidating the neural and genetic factors underlying psychiatric illness is hampered by current methods of clinical diagnosis. The identification and investigation of clinical endophenotypes may be one solution, but represents a considerable challenge in human subjects. Previously, we reported that mice heterozygous for a null mutation of the alpha-isoform of calcium/calmodulin-dependent protein kinase II (alpha-CaMKII+/-), a key molecule in synaptic plasticity, have profoundly dysregulated behaviors including a decreased anxiety-like behavior, an exaggerated infradian rhythm, and severe working memory deficit, which are similar to symptoms seen in schizophrenia, bipolar mood disorder and other psychiatric disorders. In addition, we found that almost all the neurons in the dentate gyrus (DG) of the mutant mice failed to mature at molecular, morphological and electrophysiological levels. Here we show that the mice lacking a transcription factor, Schnurri-2, exhibit abnormal behavioral pattern that is similar to that of a-CaMKII+/- mice. Schnurri-2 KO mice. Based on these results, we propose that an "immature DG" in adulthood might serve as a promising candidate endophenotype of schizophrenia and other human psychiatric disorders. Potential mechanisms underlying the "immature dentate gyrus" and strategies to normalize this phenotype will be also discussed.

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Saturday, May 15, 2010

09:00 Symposium 4: Talk ***Saturday May 15***
Kyung-An Han

Dopamine in behavioral disinhibition
Y.-C. Kim, P. Sabandal, J. Lim & K.-A. Han
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Dopamine plays pleiotropic roles in brain development and functions, and anomalous dopamine systems are associated with attention deficit hyperactivity disorder (ADHD), autism, schizophrenia, drug addiction and Parkinson's disease. One of the common behavioral traits associated with the aforementioned dopamine-related disorders is impulsivity, a type of behavioral disinhibition. To understand whether and how dopamine regulates impulse control, we employed a powerful genetic model Drosophila melanogaster and implemented a multiple object tracking system FlyTracker to monitor motor behavior. Flies defective in dopamine transporter (DAT) exhibit elevated basal locomotor activity similar to DAT mutant mice or people with ADHD. To investigate behavioral responses of DAT mutant flies to arousal stimulation, we exposed flies to ethanol. DAT mutant flies under the influence of ethanol display hyperkinetic activity (drastic movement with speed higher than 60 mm/sec) representing motor impulsivity. Remarkably, the DAT mutant's hyperkinetic behavior is sensitive to social stress. Thus, this study provides a unique system to unravel the mechanism by which the dopamine system interacts with environmental and social stimuli in impulse control. Studies are in progress to identify the underlying mechanisms.
This work is supported by the NIH/RCMI 5G12RR08124 and ABMRF/The Foundation for Alcohol Research.

09:30 Symposium 4: Talk ***Saturday May 15***
Jerry Yin

Sleep-associated dCREB2-responsive transcription
T. Tubon, J.R. Gerstner, J. Swanson, A. Tanenhaus, B. Zhang, H. Zhou, E. Gonzales, J.L. Elliott, E. Friedman & J.C.P. Yin
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Activity-dependent activation of the CREB family of transcription factors is well established, and is an integral part of modern molecular neuroscience. It is becoming apparent, at least in Drosophila, that sleep-correlated activation occurs for the dCREB2 gene. This activation has far-reaching phenotypic consequences at the behavioral (memory formation and sleep) and molecular (regulated nuclear entry) levels. In this talk, we will summarize our current understanding on how these different types of neuronal activity (awake vs. sleep) lead to activation of alternative signaling pathways, dCREB2 activation, and their different functional consequences at the behavioral level.
NIH NS063245

10:00 Symposium 4: Talk ***Saturday May 15***
Su Guo

Deciphering genetic and cellular networks controlling innate behavior in zebrafish
S. Guo
Department of Bioengineering and Therapeutic Sciences, University of California at San Francisco, San Francisco, CA 94143-2811

I will discuss our findings of two forms of innate behaviors that zebrafish display in response to environmental lighting conditions: one is a relatively simple camouflage response, and the other is a more complex choice behavior. I will present evidence that the simple camouflage response is sensitive to genetic and pharmacological manipulations including alcohol, whereas the complex choice behavior is fear/anxiety-related and engages higher brain structures similar to the mammalian amygdala. These findings establish zebrafish as a suitable model for studying mechanisms underlying emotion-related behaviors and associated human disorders.

10:30 Symposium 4: Talk ***Saturday May 15***
Josh Dubnau

Memories of a fly: Psychology, circuits, and genes
J. Dubnau, M. Cressy, H. Qin, & W. Li
Department of Neurobiology, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA

All animals appear to form memories of past experiences. The phenomenology of memory is highly conserved in terms of biochemical (genetic) signaling mechanisms. Behavioral properties of memory also are remarkably similar. And more recently, evidence has emerged that even circuit function may be similar across animals as distant as vertebrates and invertebrates. I will present recent work from my lab on a multi-level analysis of Pavlovian olfactory conditioning in Drosophila melanogaster. Our goal is to integrate findings from reductionist molecular genetic approaches, through neural circuits, through behavior.

11:30–12:30 Lecture ***Saturday May 15***
Tamara Phillips

Cool! I can breed for this!
T.J. Phillips
Veterans Affairs Medical Center, Department of Behavioral Neuroscience, Portland Alcohol Research Center, Methamphetamine Abuse Research Center, Oregon Health & Science University, Portland, OR 97239 USA

In 1980, as an undergraduate, I was fortunate to obtain laboratory research experience in the laboratory of Dr. Martin Hahn, working with mice that had been selectively bred by Dr. John L. Fuller for high or low brain size. The question we addressed was whether there are differences in learning ability associated with spurts and plateaus in brain growth and whether such differences were associated with genetically determined differences in brain size. We found that animals tested in a shock-escape T-maze did indeed show superior learning during a proposed brain growth spurt, but that the learning ability of high and low brain size mice was comparable. From this experience, I became enamored with the scientific field of behavioral genetics and have been fortunate to be trained by artisans in this field, including my graduate mentor Dr. Bruce Dudek and my postdoctoral mentor and now colleague, Dr. John Crabbe. To my surprise, much of my research has continued across my 30-year research career to utilize the particular breeding model, the selected line. Included among the lines that I have worked with or created are selected lines for differential sensitivity to ethanol-induced sedation, ethanol-induced locomotor stimulation, ethanol-induced hypothermia, ethanol-induced withdrawal severity, ethanol drinking, ethanol-induced conditioned taste aversion, methamphetamine-induced locomotor stimulation, methamphetamine-induced locomotor sensitization, and methamphetamine-induced drinking. A reverse selection project challenged the idea that all alleles relevant to the selection trait are homozygously fixed once the limits of selection have been reached and when there is no drift after selection pressure has been relaxed. But the greatest gratification of my career has been moving from the knowledge that there are genetic influences on behavior to the certainty that we will someday know what genes and gene networks increase risk or provide protection from disease. I will talk about my use of selective breeding and selected lines as powerful genetic tools in these efforts, with a focus on traits thought to be relevant to excessive drug use disorders.
Supported by the Department of Veterans Affairs, and NIH grants P60 AA010760, P50 DA018165, U01 AA013519, and R01AA11322.

Sunday, May 16, 2010

Carl Faingold

Neuronal network for audiogenic seizures in the Genetically Epilepsy-Prone Rat (GEPR-9) - molecular mechanisms of network expansion

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The two substrains of genetically epilepsy-prone rats were derived from the Sprague-Dawley strain, and they exhibit consistently different degrees of audiogenic seizure (AGS) severity in response to acoustic stimulation. AGS in the severe seizure strain (GEPR-9s) end in tonic hind limb extension, and in the moderate seizure strain (GEPR-3s) the seizures end in generalized clonus. The strains are genetically distinct, since they differ from each other at 74 of 107 genetic markers examined. Mechanistic studies in GEPR-9s have observed a deficit of GABA-mediated inhibition, particularly in inferior colliculus, which is due to reduced effectiveness of exogenously applied and endogenously evoked GABA-mediated inhibition. Thus, when GABA was applied directly onto inferior colliculus neurons of GEPR-9s, significantly more GABA was required to produce the same degree of firing reduction as compared to Sprague-Dawley rats. Acoustically-induced inhibition of inferior colliculus neurons has been shown to be mediated by GABA, and the degree of inhibition produced by three different forms of acoustic stimuli was found to be significantly less in GEPR-9s as compared to Sprague-Dawley rats. Studies, using anatomical, focal microinjection, and single unit recording techniques, indicate that the requisite structures for the AGS neuronal network in GEPR-9s lie in the brainstem and that a hierarchy of activation occurs in neurons in these network nuclei during seizures. Thus, the inferior colliculus is the consensus seizure initiation site. The wild running behavior is driven from the deep layers of superior colliculus, and clonus and tonus are driven by the periaqueductal gray and pontine reticular formation, which are gated by the substantia nigra retriculata. In response to 7-14 days of AGS repetition (AGS kindling) long-lasting increases in seizure duration and additional seizure behavior occur, and the AGS neuronal network expands from the brainstem to include forebrain sites, particularly the amygdala. The kindled AGS behaviors closely mimic the pattern seen in human tonic-clonic (grand mal) epilepsy. AGS kindling induces NMDA receptor-mediated changes in the amygdala, since focal microinjection of NMDA in un-kindled GEPR-9s can mimic AGS kindling but recovery occurs by 24 hr. The permanence of AGS

kindling appears to be triggered by the NMDA receptor-triggered molecular cascade that involves cAMP, which is implicated as a critical mechanism subserving the long-lasting seizure changes. Thus, when a forskolin derivative that increases cAMP production (MPB forskolin, 25-100 pmol/side) is administered focally into the amygdala of un-kindled GEPR-9s, a long-lasting (> 5 weeks) kindling-like seizure behavior change is induced. Bilateral focal microinjection into the amygdala of AGS-kindled GEPR-9s of an agent that inhibits the action of cAMP (SQ22,536, 0.25 and 0.50 nmol/side) reversed the effects of AGS kindling. The mechanisms that mediate AGS kindling in GEPR-9s may be relevant to the progressive seizure changes that often occur in epileptic patients.

NINDS and Excellence in Academic Medicine / SIU School of Medicine

Krushinsky-Molodkina (KM) inbred rat strain: audiogenic epilepsy, catalepsy, inheritance

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Identifying animal models that recapitulate human epilepsies is believed to be of medical importance for studies of anticonvulsants, for dissection of molecular and biochemical pathogenesis of epilepsy, and for search on epilepsy susceptibility genes. Apart from its practical importance, the rodent audiogenic epilepsy is an enigmatic biological and genetic phenomenon. Actually the biological significance of high sound sensitivity for rodent survival was never questioned but at the same time its connection with audiogenic epilepsy was not analyzed either. Several audiogenic rat strains exist, and rat audiogenic seizures had been explored by different research groups. In 1948 L. Krushinsky, L. Molodkina and D. Fless started the selection of rats for high susceptibility to “sound seizures” in Moscow University. Starting from mid-1950-s the KM strain was extensively used as an epilepsy model in pharmacology and as a model of the catatonic state. In 1986-87 the inbreeding of KM strain was initiated, and proceeds to date. The convulsive seizure develops in almost 100% of KM rats with wild run onset in 2-3 sec and the clonic-tonic seizures developing in 7-9 sec. There is a marked phenotypical similarity of the audiogenic seizures in rat strains in Russia, United States, China, and Brazil, including the specific pattern of seizure stages, audiogenic kindling and post-ictal depression, brain metabolite levels and their changes after the audiogenic seizure. Audiogenic seizures are initiated in brain stem structures, while hippocampus and neocortex do not appear to be involved. However, in audiogenic kindling, which develops after numerous daily sound exposures, epileptiform excitation is seen in the neocortex and hippocampus. Genetic research of audiogenic seizures in mice identified several genes which influence strongly this phenotype. The heritability of this trait in rats is more difficult to determine. In KM rats the diallelic cross showed that audiogenic seizure heritability is polygenic with additive effects, and alleles determining the resistance to sound induced epilepsy were dominant. Later the attempt to select against audiogenic seizure susceptibility was initiated, and it proceeds up to the present time. F2 hybrids from the cross of Wistar female rat, resistant to audiogenic seizures, and KM males which were resistant to sound epilepsy were backcrossed to KM

rats in two generations and then maintained as an outbred strain with permanent selection for the absence of audiogenic seizures in response to loud sound, which is now in the S 17 generation. In spite of permanent selection against the trait only 35-45% of rats of new strain are not susceptible to audiogenic seizures, and we are currently unable to explain this phenomenon. On the basis of hybrid data the new digenic model of rat audiogenic seizure susceptibility control was developed with incomplete penetrance of genotypes. (Supported by Russian Foundation for Basic Researches (07-04-00481), by grants from Swiss National Foundation (NN 7IP 051224, IB74BO-111081).

10:00 Symposium 5: Talk *Sunday May 16*
Noberto Garcia-Cairasco

Epilepsy and Neuropsychiatric Comorbidities: Lessons from the Wistar Audiogenic Rat (WAR) Strain
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We developed the Wistar Audiogenic Rat (WAR) Strain by genetic selection of Wistar progenitors susceptible to audiogenic seizures. Currently, we are near the 50th generation of sisters x brothers inbreeding. Most of the research that has been made with the WARs is related to several aspects of the epilepsies. In fact, the WARs represent a very strong avenue of research to increase our knowledge on ictogenesis and epileptogenesis, and for the development of new anti-epileptic drugs. Our neuroethological, EEG and cellular studies have shown that the expression of acute audiogenic seizures, a model of tonico-clonic seizures, depends on the activation of brainstem networks, among them, the inferior and superior colliculi, the central grey and the substantia nigra. Chronic audiogenic seizures, the so-called audiogenic kindling, are a model of temporal lobe epilepsy (TLE), expressed as behavioral and EEG limbic seizures, with strong participation of cortex, amygdala and hippocampus. Besides innate alterations in naïve WARs, in kindled audiogenic seizures we have shown behavioral, plastic and molecular alterations, such as up-regulation of specific genes, compatible with epileptogenesis. However, we have also discovered that an innate decreased body growth of the WARs is associated to altered hypothalamus-pituitary-adrenal axis, including adrenal gland hyperplasia. Further alterations such as hypertension and increased sympathetic tonus make the WARs a suitable model to study sudden unexpected death in epilepsy (SUDEP). As a whole, these endocrine and cardiovascular alterations are in synchrony with the decrease of exploration of both the open arms of the elevated plus maze and the open field, a signature of WAR endogenous anxiety. In addition to be more sensitive to chemical and electrical convulsant treatments, WARs are also suitable to study auditory function and pathologies such as tinnitus and deafness. In the latter case we are studying the potential overlapping, synergism and eventual antagonism between circuits for epilepsy, fear-potentiated startle and the formation of auditory phantoms (tinnitus). We use behavioral, electrophysiological, cellular and molecular approaches to evaluate the mechanisms associated to these phenotypes. What is

important to understand is that the genetic background of the WARs associated to their, especially chronic seizure experience, is modeling well-known conditions such as limbic kindling, chronic conditioned fear and chronic tinnitus. Those conditions usually progress to cognitive, mood, emotional and, in general, neuropsychiatric health-harming entities.

In conclusion, the WARs were selected originally and used mostly for epilepsy studies. However with the progression of studies, we detected a wide range of new behavioral, cellular and molecular alterations, certainly associated to their pattern of genetic selection, including aspects of gene linkage and development of what we seen as compensatory traits. For those reasons, we are currently proposing that the WAR strain can be explored as a reliable genetic model of epilepsy-neuropsychiatry comorbidities.
Funded by Brazilian Foundations FAPESP; FAPESP-Cinapce; CAPES-PROEX, CNPq and FAEPA/HC-FMRP-USP.

Altered Voltage-Gated Calcium Channels and Signaling and Inherited Seizure Susceptibility in the Genetically Epilepsy-Prone Rat (GEPR-3)

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Genetic factors are known to play important roles in the etiology of generalized tonic clonic seizures in humans. Inherited or genetic models of epilepsy therefore provide unique opportunities to study the underlying mechanisms of seizure predisposition in models that unlike normal brains do not require exposure to exogenous seizure-inducing treatments. One model of interest is the genetically epilepsy-prone rat (GEPR), which displays inherited susceptibility to acoustically evoked generalized tonic-clonic seizures. In the GEPR and in most patients the genetic predisposition to seizures shows complex inheritance such that more than one gene leads to epilepsy syndrome. In certain human epilepsies a genetic component that predispose to seizures is known, including ion channel abnormalities. The genetic factors that predispose the GEPR to seizures remain poorly understood. Neuronal network studies have determined the brain sites critical to epilepsy in the GEPR, and mechanistic studies in these sites may provide future target for ion channel abnormality studies. Multiple lines of evidence indicate that the inferior colliculus (IC) is critical in the initiation of acoustically evoked generalized tonic-clonic seizures in the GEPR. More precisely, electrophysiological studies revealed that at the transition to seizures, IC neurons exhibited sustained increases in neuronal firing. Intrinsic properties of neurons are critical in the generation of epileptiform bursts. The moderate seizure strain (GEPR-3s) exhibit sound-induced seizures that end in generalized clonus. In the IC of GEPR-3s, voltage-gated Ca2+ channels play an important role in neuronal hyperexcitability that leads to seizures. Pharmacological studies reported that Ca2+ channel antagonists suppressed acoustically evoked seizures in the GEPR-3s providing a causal relation between voltage-gated Ca2+ channels and inherited seizure susceptibility. Accordingly, the current density of L-, N- and R-type of high threshold voltage-activated (HVA) Ca2+ channels was markedly increased in IC neurons of the GEPR-3 compared to control Sprague-Dawley rats. Rapid confocal microscopy reveals that the increased HVA current density translates into elevated K+-depolarization-evoked intracellular Ca2+ transients in IC neurons of the GEPR-3. Furthermore, molecular

studies show that seizures susceptibility is associated with upregulation of CaV1.3 (a1D, L-type) and CaV2.3 (a1E, R-type) in IC neurons of the GEPR-3. A single seizure enhances total HVA current density and selectively upregulated CaV2.1 (a1A, P/Q-type) in IC neurons of the GEPR-3. Thus, upregulation of CaV1.3 and CaV2.3 may contribute to the genetic basis of the enhanced seizure susceptibility in GEPR-3s. There is a need for extensive future studies to identify genes encoding for these ion channels that contribute to seizure susceptibility in the GEPR.

11:30 Open Talk *Sunday May 16*
Igor Ponomarev

Transcriptional networks in brains of alcoholic and nonalcoholic individuals

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Alcohol abuse causes widespread changes in gene expression in human brain, which may contribute to the development and maintenance of alcohol dependence. To identify key genes and molecular pathways contributing to alcoholism, we profiled gene expression levels of 33 postmortem human brains (3 regions of the amygdala and superior frontal cortex; 16 controls and 17 alcoholic cases) using Illumina microarrays. We first used a weighted gene coexpression network analysis (WGCNA; Zhang and Horvath, Stat Appl Genet Mol Biol, 2005), which is based on gene-by-gene correlations across all samples, to identify modules of coexpressed genes based on all detected transcripts. We then identified alcohol-related modules, i.e., modules significantly enriched for genes differentially expressed between alcoholics and controls. Functional over-representation analysis was then applied to examine organizing principles of alcohol-related modules. In general, genes were clustered into modules according to their involvement in different physiological processes, functional groups, chromosomal locations and cell types. We identified both brain region-specific and common alcohol-related changes. Modules enriched with cell type-specific genes were mainly region-specific, while examples of common changes included genes located on chromosome 19 and genes involved in immune/inflammatory responses. Two “hub” genes, i.e., genes with highest degrees of connectivity to other genes within their modules, which were differentially regulated between alcoholics and controls in all three regions of the amygdala and were involved in NMDA receptor signaling, were nominated as candidates for functional validation. Our transcriptional network analysis integrated multiple levels of data and connected molecular pathways to nervous system function and brain pathology. This approach provides contextual information about biological function that may not be available when examining single genes and assists in formulation of new refined hypotheses. Funding Support: NIH, NIAAA grants: AA012404 to AH, INIA grants (AA013518 to AH, AA016648 to RDM, AA013517 Pilot Projects to RDM and IP, AA013476 Subcontract to IP)

11:50 Open Talk *Sunday May 16*
Angela Ozburn

Clock∆19 mutants exhibit increased ethanol preference and consumption

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Recent studies point to a link between abnormal or disrupted circadian rhythms and drug addiction. Additionally, studies identify roles for circadian genes in the regulation of drug sensitivity and reward. Mice with a dominant negative mutation in the Clock gene (Clock ∆19 mutant mice) show an increased sensitivity to cocaine. To extend these studies, we assessed whether the core molecular Clock gene has a role in alcohol intake. We measured ethanol preference and consumption in Clock∆19 and wild-type mice using the continuous access two bottle choice paradigm (n=10-11/genotype). Escalating ethanol concentrations were offered versus water for two days each, starting with 3% ethanol (v/v in tap water) and continuing with 3% increases up to 21% ethanol. To determine the effect of genotype and ethanol concentration offered, a two-way ANOVA was performed for ethanol preference and consumption data. Clock ∆19 mutant mice exhibited increased ethanol preference as compared to wild-type mice. Ethanol preference was dependent on genotype and ethanol concentration offered (genotype x concentration interaction $p < 0.05$; main effect of genotype $p < 0.05$; main effect of concentration $p < 0.001$). Clock ∆19 mutant mice exhibited increased ethanol consumption as compared to wild-type mice. Ethanol consumption was dependent on genotype and ethanol concentration offered (genotype x concentration interaction $p < 0.01$; main effect of genotype $p < 0.05$; main effect of concentration $p < 0.001$). Interestingly, Clock ∆19 mutant mice exhibited significantly higher ethanol preference and consumption at higher ethanol concentrations (as revealed by Bonferroni post-hoc analysis). Total fluid consumption did not differ between wild-type and Clock ∆19 mutant mice. Studies generally show that very few genetically modified mice exhibit increased alcohol intake. In addition to Clock ∆19 mutants, mice with a null mutation in the circadian gene Per2 also exhibit increased alcohol intake. Per2 expression is regulated by the transcription factor CLOCK. These results suggest an important role for Clock in alcohol intake. Future studies include characterization of ethanol-related behaviors in Clock ∆19 mutant mice, as well as in other circadian mutants.

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12:10 Open Talk ***Sunday May 16***
Christopher Kliethermes

Food deprivation induces strain-specific behavioral responses to ethanol in Drosophila melanogaster
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Ernest Gallo Clinic & Research Center, Department of Anatomy and Program in Neuroscience, University of California, San Francisco

Food deprived animals show faster acquisition of operant behaviors whether the reinforcer is food or a drug of abuse, and also show elevated basal and drug-stimulated locomotor activity, implying common neural circuits underlie these behaviors. To determine whether genetics might influence this trait, a panel comprising genetically segregating and inbred fly strains was tested for ethanol-stimulated locomotion following an overnight food-deprivation. This protocol resulted in a body weight reduction to approximately 90% of free-feeding levels averaged across all strains. Compared with fed flies, food deprived flies showed elevated basal and ethanol-stimulated locomotor activity. Strain-specific effects were also observed, with some strains showing robust food deprivation-induced increases in locomotion, and other strains that appeared unaffected. Because baseline locomotion was strongly phenotypically correlated with ethanol-stimulated locomotion, the area under the ethanol-stimulated locomotion curve was regressed onto the average basal locomotor rate to obtain a residual score. As with the uncorrected score, strain specific effects of food-deprivation were still found on this residual score, indicating that food deprivation results in a strain specific augmentation of ethanol-stimulated locomotor activity.
This research was supported by funds from the State of California for medical research and NIH grant AA16876

12:30 Open Talk ***Sunday May 16***
John Crabbe

Alcohol and tastant preference drinking in mice selectively bred for High Drinking in the Dark (HDID-1)

J.C. Crabbe, S.E. Spence, L.L. Brown, A.J. Cameron, J.P. Schlumbohm, C.-H. Yu & P. Metten
Portland Alcohol Research Center, Department of Behavioral Neuroscience, Oregon Health & Science University, and VA Medical Center, Portland, OR USA

We have selectively bred mice that achieve high blood ethanol concentrations (BECs) during a limited period of access to ethanol. When offered a single bottle of 20% ethanol v/v for 4 hrs starting 3 hrs after the onset of their circadian dark period, High Drinking in the Dark (HDID-1) mice drink enough ethanol to achieve average BECs of nearly 100 mg%, and more than 60% of them exceed this level. This “Drinking in the Dark” phenotype is genetically correlated with standard two-bottle ethanol preference across numerous inbred strains, suggesting influence of some genes on both traits. We compared mice from the HDID-1 selected line with the HS/Npt genetically heterogeneous stock that served as the foundation for the selective breeding project. We serially offered mice access to 3, 6, 9, 12, 15, 20, 25, 30, 35, and 40% ethanol in tap water vs tap water. Both solutions were freely available at all times and each was offered for 4 days. Bottle positions were changed each 2 days. There was no difference between HDID-1 and HS/Npt controls in two-bottle preference drinking for all ethanol concentrations up to 20%. At the highest concentrations, the HS/Npt mice drank more than the HDID-1 mice. Absolute intake of ethanol (g/kg/day) increased as concentration offered increased, in both genotypes. Females drank more than males in both genotypes. We then offered the same mice two concentrations each of three tastants (quinine, sucrose, saccharin) using the same method. We found that after experiencing the ethanol drinking series, neither genotype showed either preference or avoidance for any tastant. Therefore, we also compared naive HDID-1 and HS/Npt mice fore tastant preference. Results suggest at least partial genetic independence of ethanol Drinking in the Dark from two-bottle ethanol preference and preference for tastants.

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14:00-15:00 Lecture ***Sunday May 16***
Hiroki Ishiguro

Analysis of NrCAM related molecular pathway underlying addiction

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Genomic analysis revealed that a haplotype associated with decreased NRCAM expression in brain is protective against addiction vulnerability for polysubstance abuse in humans. Nrcam knockout mice do not develop conditioned place preferences for morphine, cocaine, amphetamine, and alcohol. These results imply that NrCAM may have a role in a common pathway underlying polysubstance abuse. In order to gain insight into NrCAM involvement in addiction vulnerability, which may involve specific neural circuits underlying behavioral characteristics relevant to addiction, we evaluated several behavioral phenotypes in Nrcam knockout mice. Consistent with a potential general reduction in motivational function, Nrcam knockout mice demonstrated less curiosity for novel objects and for an unfamiliar conspecific, showed also less anxiety in the zero maze, open field, or passive avoidance test. Nrcam heterozygote knockout mice reduced alcohol preference and buried fewer marbles in home cage. NrCAM related molecules were also screened in NRCAM downregulated tissues, siRNA treated CHO cells and brain tissues of Nrcam knockout mice. Several candidate molecules downregulated synchronously with NRCAM had been reported as candidate genes for alcoholism and depression. Inhibitor for one of the molecules induced similar behavioral effects in C57B/6J inbred mice. These observations provide further support for role of NrCAM related molecules in substance abuse including alcoholism vulnerability, possibly through its effects on behavioral traits that may affect addiction vulnerability, including novelty seeking, obsessive compulsion and responses to aversive or anxiety-provoking stimuli.
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Ministry of Health, Labour and Welfare, and Grant support from the Japan Brain Foundation (Japan).

15:30 Open Talk ***Sunday May 16***
Emmanuel Onaivi

CNS effects of CB2 cannabinoid receptors
E.S. Onaivi, H. Ishiguro, Q.-R. Liu, J.-P. Gong, P.A. Tagliaferro, A. Brusco, & G.R. Uhl.
William Paterson University, Wayne, NJ, USA, IRP-NIDA-NIH Baltimore, MD, USA, Ikeda Hospital, Happoukai Medical Corporation, Ryugasaki, Japan, Universidad de Buenos Aires, Argentina.

Endocannabinoids, cannabinoids and marijuana use activate two well characterized cannabinoid receptors (CBRs), CB1-Rs and CB2-Rs. Previously, it was thought that CBRs in CNS were predominantly the CB1-Rs and that CB2-Rs were expressed in peripheral and glia tissues. Therefore, the expression of CB1-Rs in the brain and peripheral tissues has been well studied and characterized, but neuronal CB2-Rs have received much less attention than CB1-Rs. There is however mounting credible evidence for the functional neuronal CB2-Rs in the CNS. Here, we used immunoblotting, genotyping, immunoelectron microscopy, mouse behavioral assessment and quantified human and rodent CB2-R specific isoforms in different tissues and brain regions as well as in mice treated with CBR ligands. Association studies were performed between CB2-R SNPs in schizophrenia and depression in human population. We discovered the peripheral and CNS CB2-R subtype specific expression patterns in human and rodents that resolved the ambiguity and controversy over the neuronal expression of CB2-Rs that are localized mainly in post-synaptic elements of rat hippocampus and substantia nigra. Species comparison found that the CB2-R gene of human, rat and mouse genomes deviated in their gene structures and isoform expression patterns. Naive BTBR mice that have been reported to have autism-like behavioral phenotypes have an upregulated high level of CB2A gene expression in the cerebellum. There is an increased risk of schizophrenia for people with low CB2 receptor function. Indeed, our studies provide the first evidence for the neuronal CNS effects of CB2-Rs and its possible involvement in drug addiction and neuropsychiatric disorders. Thus the results provide much improved information about CB2 gene structure and its human and rodent variants that should be considered in developing CB2-R-based therapeutic agents.
Supported by NIDA-NIH, WPUNJ.

15:50 Open Talk ***Sunday May 16***
Elena Jazin

Regional differences in sexually dimorphic gene expression in adult mice brain
B. Reinius[1], K. Kullander[2], G. Rosen[3], L. Lu[4], R. Williams[4] & E. Jazin[1]
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The presence of extensive sexual gene-expression dimorphism in the brains of humans, other primates and mice is now recognized. Previous genome-wide expression studies investigated the whole brain or one single brain region. Here we report a comprehensive study of 480 microarrays analysing genome-wide expression in diverse anatomical structures of the mouse brain, including neocortex, striatum, hippocampus and eye from 240 male and 240 female samples. Lung is also included as a peripheral tissue contrast**.** Our investigations show specificity in the amount and type of genes with sexual dimorphism in different parts of the brain. For example, applying stringent selection criteria, we identify 173 sex-biased genes in striatum, which are encoded in many different chromosomes. In contrast, using the same criteria, we identify a low amount of sex-biased genes in neocortex (17), hippocampus (12) and eye (22), most of which are encoded on sex chromosomes. A group of these sex-linked genes are biased in the same way on several of the tissues investigated. These shared genes encode Y-chromosome located genes, as well as X-chromosome encoded transcripts known to escape inactivation in the silenced X-chromosome. Alongside with them, we identify five novel X-linked transcripts with up-regulation in several female tissues. Interestingly, known and novel X-genes up-regulated in females are localized as close pairs on the X-chromosome. This raises the possibility of a collective mechanism for escape from X-inactivation.

16:10 Open Talk ***Sunday May 16***
Amy Eisener-Dorman

Using comparative analysis and haplotype mapping approaches to identify quantitative trait loci in closely related strains
A.F. Eisener-Dorman[1], J.S. Bailey[1], L. Grabowski-Boase[3], B.M. Steffy[2], T. Wiltshire[2] & L.M. Tarantino[1]
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The use of inbred strains of mice that are both phenotypically and genetically divergent is standard practice in QTL mapping experiments. However, transgressive segregation can result in the identification of QTL in crosses between strains that are genetically or phenotypically similar. The availability of a dense SNP map in over 70 inbred strains of mice now makes QTL studies in closely related inbred strains possible. We have conducted two QTL studies of open field behavior in both a C57BL/6J (B6) X C58/J (C58) F2 intercross and inter- and backcross animals derived from B6 and C57L/J (C57) founder strains. Both of these QTL studies identified several significant QTL despite the phenotypic and/or genetic similarity displayed by the parental strains. Comparative analysis of both QTL crosses indicates that there are both common and cross-specific QTL. The observation of cross-specific QTL provides an opportunity to use haplotype comparisons to narrow the QTL regions. We examined haplotype sharing among these three inbred strains and were able to reduce a QTL interval on Chr 9 by 90%, resulting in a QTL region of 5.5Mb. This work highlights the ability to identify QTL in closely related inbred strains and to take advantage of the genetic similarities between these strains to significantly narrow QTL regions.
NIH NIDA Grant DA 022392 to L.M.T. and a Novartis Grant SFP-1406 from the Genomics Institute of the Novartis Research Foundation

16:30 Open Talk *Sunday May 16*
Robert Williams

A whole genome sequence for DBA/2J and its use in reverse complex trait analysis
X. Wang, R. Agarwala, T. Capra, D.M. Church, D.C. Ciobanu , D. Li, L. Lu, K. Mozhui, M.K. Mulligan, S. Nelson, K.S. Pollard, W.L. Taylor, D.B. Thomason, R.W. Williams
University of Tennessee Health Science Center; NCBI NIH; University of Nebraska; University of California at Los Angeles; Gladstone Institute

Complex trait analysis is a forward genetic method that begins with phenotypes for often large sets of genetically diverse cases. Heritable variation of these phenotypes is then mapped to generate sets of quantitative trait loci (QTLs) that can be distributed across the whole genome. The final and most difficult step involves testing the possible function of single gene variants located within each QTL. In contrast, reverse genetic methods start with well defined sequence variants--usually knockout alleles--and test their roles in higher order phenotypes.

High-throughput sequencing is blurring the distinction between these complementary approaches. It is now practical to generate complete lists of DNA variants segregating in intercrosses, recombinant inbred panels, and congenic and consomic strains. The effects of DNA variants can be studied effectively in these segregating populations using reverse genetic methods, a method called reverse complex trait analysis.

We are testing this approach using a family of 80+ recombinant inbred mouse strains (BXD) that were made by crossing inbred strains C57BL/6J (B6) and DBA/2J (D2). B6 was used to generate the mouse reference genome (NCBI Build 37.1). D2 was sequenced by our group using four paired-end libraries (200-4000 bp inserts) and two high throughput sequencing platforms (SOLiD 3 and Illumina GAII). Sequence coverage is sufficiently dense (~56-fold) that we are able to define most SNPs, indels, and CNVs segregating in any cross that involves B6 and D2 parents (BXDs, B6.D2 congenic, or B6D2 intercrosses). We have extracted ~4.5 million SNPs, ~550,000 indels, ~20,000 CNVs in the range of 1 Kb to 100 Kb, and thousands of inversions. All of these polymorphisms are by definition “common” with close to an optimal allele frequency to detect functional repercussions. A small number of sequence variants--including premature stop codons and frame-shift mutations--are likely to produce major differences

among BXD strains. These mutations can be studied using methods that are similar to the analysis of KO lines, but with the added benefit that putative phenotypes linked to mutations can be mapped back to a gene locus to confirm causality. We have used a large database of classical phenotypes in this reverse analysis of DNA variants. We have also exploited whole transcriptome expression data for multiple brain regions (array and RNA-seq) to study possible effects of DNA variants on RNA expression, brain structure, and behaviors.

Reverse complex trait analysis is a potentially powerful approach. One of the major statistical issues is control for false discovery associated with the large number of reverse genetic tests. Bayesian methods that exploit multiple independent data sets are often essential. Another inevitable challenge is linkage disequilibrium of potentially large numbers of sequence variants. Careful analysis of haplotype structure and the use of partial correlation and structural equation models are proving helpful in testing causality.

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POSTER ABSTRACTS
(Friday May 14, 2010)

P-01
Robert Benno

Gene x environment interaction in the development of Autism Spectrum Disorders in the mouse: Potential interplay between immune activation and the DISC1 mutation

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Autism spectrum disorders (ASD) are a complex group of diseases believed to be due to a multitude of factors. Recent studies have suggested the use of a gene x environment x development interaction (GEDI) approach may be necessary to understand the mechanism behind this disorder. Several studies using the BTBR T+tf/J inbred mouse have shown that this strain may serve as a model genetic system for ASD (Crawley et al., 2007). It has been suggested that the BTBR mouse meets all of the three defining symptoms of autism: poor social communication, minimal social interaction, and repetitive behaviors typically involving interaction with a limited number of objects.

In our laboratory we have begun a series of experiments utilizing three inbred mouse strains (BTBR T+ tf/J, 129S1/SvImJ, and C57BL/6J) in an attempt to understand the mechanism by which the ASD phenotype develops in the BTBR strain. We chose these three strains based on: the presence of a 25 base deletion in the DISC1 gene (both BTBR and 129S1) and an abnormal immune response in the BTBR strain. We hypothesized that full expression of the psychopathology shown to be associated with the DISC1 deletion requires an immune activation. Thus we injected all three strains of mice with the viral mimic Poly I:C (5 or 10 mg/kg IV) on day 9 of gestation. Our expectation was that the offspring of the BTBR strain would show little or no response, the 129S1 strain would show profound effects, and the C57 strain would show some ASD like behavioral effects as previously reported. We utilized several behavioral measures to test our hypothesis including: isolation induced ultrasonic vocalizations during the postnatal period, maternal-pup retrieval, juvenile social behavior, plus maze, and marble burying. Preliminary data suggests that there is a gene x environment interaction in our model for several of the behavioral phenotypes.
Funded by William Paterson University

P-02
D. Caroline Blanchard

BTBR T+tf/J mice show social deficits in seminatural Visible Burrow Systems

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Autism spectrum disorders (ASD) are a group of increasingly prevalent neurodevelopmental disorders defined by social interaction and communication deficits and ritualistic-repetitive behaviors. They show extremely high concordance between monozygotic twins, but likely involve an interaction between multiple genes and possible environmental factors during development. As their primary diagnostic indices are behavioral, animal models need to demonstrate social deficits related to core ASD symptoms. Previous studies on BTBR T+tf/J (BTBR) mice have shown a range of social deficits, as well as high levels of self-grooming, in tasks specifically designed to evaluate these behaviors.

The Visible Burrow System (VBS) is a semi-natural habitat in which groups of mice or rats live for extended periods in situations affording “burrows” based on those constructed in nature, as well as “open space”; maintained under a 12:12 hr. light/dark cycle. Same-strain groups of 3 male BTBR or C57Bl/6J (B6) mice were maintained in VBS for 4 days, with video recordings of each group for 24 hrs/colony (total = 192 hrs) over this period. Time samplings of behaviors indicated that BTBRs showed an extremely robust pattern of reduced approach, huddling, allogrooming, and chase/follow, with enhanced flight, alone, and self-grooming. These findings are in agreement with earlier data on the BTBR mice, and validate their reduced sociality and enhanced self-grooming in a semi-natural situation in which behaviors and a behavioral time-budget are self-generated.
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P-03
Stephen Boehm II

Adolescent and Adult High Alcohol Preferring Selectively Bred Mice Engage in Binge-Like Alcohol Intake Using Drinking in the Dark Procedures

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Adolescent binge alcohol drinking is a major health concern in the United States, accounting for up to 69% of drinking among youth aged 12-18 years in 2003 (Miller et al., 2008). The development of useful animal models is crucial if we are to understand the neurobiological underpinnings of adolescent binge alcohol drinking. We recently reported that adolescent C57BL/6 (B6) mice consume significantly more ethanol than their adult counterparts when presented using Drinking in the Dark (DID) procedures (Moore et al., 2010). DID results in consumption of high intoxicating amounts of a 20% ethanol solution in B6 mice, producing blood ethanol concentrations (BECs) in excess of 80 mg/dL in just two hours. In the present study we wished to determine whether adolescent High Alcohol Preferring (HAP) mice also consume binge-like amounts of ethanol, and whether such intake exceeds that of their adult counterparts. Male and female adolescent (P30) and adult (P90) HAP mice were allowed daily access to an 20% ethanol solution or plain tap water for two hours each day (3 hours into the dark cycle) for 15 days. On days 3 and 15 homecage locomotion was assessed during the two hour access period, and balance beam motor impairment was determined immediately afterwards. On day 16, all animals were challenged with ethanol (1.75 g/kg; IP), returned to the homecage for 10 min during which locomotor activity was assessed, and tested on the balance beam apparatus immediately thereafter. Ethanol intakes generally increased over days, particularly for adolescents, but did not differ by sex or age, ranging from 2.1-3.7 g/kg on day 3, and 4.1-4.3 g/kg on day 15. This level of intake by adolescent mice was associated with a significant enhancement in homecage locomotion on day 3, but not day 15. Balance beam motor impairment was not altered in any group on either day. Although BECs were not determined on days 3 or 15, BECs on day 9 indicated that adolescents and adults of both sexes consumed physiologically relevant amounts of ethanol, with group means ranging from 68-127 mg/dL. Importantly, these BECs significantly correlated with intake on day 9. Finally, although day 16 ethanol challenge did not differentially alter homecage

locomotor activity, animals previously allowed daily binge-like ethanol access developed significant tolerance to ethanol's motor impairing actions as assessed using the balance beam apparatus. These results demonstrate that adolescent HAP mice will consume physiologically relevant amounts of repeated binge-like ethanol, ultimately producing tolerance to ethanol's motor impairing actions, but that this intake does not differ from that of adult HAP mice. Ongoing work is aimed at determining whether adolescent ethanol exposure alters subsequent binge-like ethanol intake in adult HAP mice.

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P-04
Peter Clark

An analysis of c-Fos, Arc, and Zif268 induction in new and pre-exisiting dentate granule neurons from acute bouts of voluntary running in C57BL/6J mice

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The functional significance of newly formed granule neurons in the adult mammalian hippocampus remains a mystery. Recent studies have used the expression of immediate early genes (IEG) such as c-Fos, Zif268, and Arc to demonstrate that new granule neurons become activated in association with performance on hippocampal-dependent cognitive tasks. It has been hypothesized that these new neurons become activated because they are involved in hippocampal-dependent learning and memory retrieval. However, most studies have not controlled for effects of acute bouts of locomotor activity on stimulating IEG expression while performing the cognitive task. For instance, mice housed in cages with running wheels display a massive increase in c-Fos expression in the granule cell layer that is strongly related to the distance the animal was running 90 minutes before euthanasia. Further, we have recently reported that new granule neurons preferentially display c-Fos in response to acute bouts running as compared to older granule neurons. The purpose of this study is to determine the extent to which Arc and Zif268 expression are also strongly up-regulated in response to wheel running in both pre-existing and newly formed neurons, as well as identify which IEGS are most strongly influenced by acute locomotor activity. Therefore, 7 week old C57BL/6J female mice were placed in cages with (n=10) or without (n=6) running wheels for 30 days. The first 10 days, mice received daily injections of BrdU (50mg/kg) to label dividing cells. On day 31, animals were euthanized by transcardial perfusion during peak wheel running activity. Immunohistochemistry is currently being performed on free floating sections with antibodies against BrdU (to label new cells), NeuN (to label mature neurons), c-Fos, Arc, and Zif268 (to label IEGs). The predicted outcome is not clear. One possibility is that all IEGs will be induced from acute running and that new neurons will be recruited into this IEG induction equally. An alternative is that some of the IEGs will not be regulated by acute running. Moreover, new neurons may be recruited into some of the IEG responses but not others. These results will provide important information to help further understand the functional significance of new neurons in behavior. Funded by NIH.

P-05
Todd Darlington

Voluntary alcohol use and exercise influence differential gene expression in mesocorticolimbic dopamine pathway

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Prior research has revealed a relation between natural reward seeking behavior and drugs of abuse at the behavioral and neurobiological levels (Nestler, 2005). Our laboratory recently reported that in C57Bl/6Ibg mice, preference for alcohol decreases when there is an exercise wheel present (Ehringer et al. 2009). Given that the release of dopamine in the nucleus accumbens is a crucial step in signaling reward that has been implicated in both alcohol and exercise behaviors (Di Chiara and Imperato, 1985, Dishman et al. 2006), we measured mRNA expression of two genes important in regulating this pathway, tyrosine hydroxylase (Th) and dopamine receptor D2 (Drd2). We hypothesize that changes in gene expression will be influenced by different environmental stimuli (alcohol, exercise, both, or neither). Adult female C57Bl/6Ibg mice were housed individually in one of four cage conditions: empty with water, empty with alcohol and water, running wheel with water, or running wheel with alcohol and water. After 13 days, mice were sacrificed and the cortex, striatum, and midbrain were collected. Total RNA was extracted, reverse transcribed, and TaqMan¬Æ probes for Th and Drd2 were used for real-time quantitative PCR with an endogenous control (Gapdh). Preliminary results suggest that midbrain expression of Th is increased in response to both alcohol and exercise, but alcohol may attenuate the increase in response to exercise when both are present. In the cortex and striatum, Drd2 expression is decreased in response to alcohol, exercise, or both. Results from this research should provide improved understanding of the neurobiology of alcohol use and exercise. This may lead to improvements in the prevention and treatment of alcohol disorders in humans.
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P-06
Karen Brebner

Pretreatment with the HDAC inhibitor valproic acid attenuates cocaine-induced sensitization: Behavioural and molecular effects
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One of the hallmarks of drug addiction is continued drug craving and the risk of relapse to drug use, despite long-term drug abstinence. Currently, little is known about how drug exposure causes changes in behaviour that persist for months or years after use. Transient alterations in gene expression can be observed in localized brain regions after both acute and repeated cocaine administration. Increasing evidence suggests, however, that psychostimulants may induce epigenetic changes in gene expression, which could contribute to the chronically relapsing behaviour that is characteristic of drug abuse. Epigenetic changes are self-maintaining modifications of DNA and chromatin that can be elicited through a number of mechanisms including histone acetylation. The removal of acetyl groups from the N-terminal sequences of histone proteins is catalyzed by the enzyme histone deacetylase (HDAC). In this study the effects of HDAC inhibition on the development of behavioural sensitization to cocaine was investigated. The hypothesis was that sensitization to cocaine causes an epigenetic imprint and that modifying this imprint using the HDAC inhibitor valproic acid (VPA) would modify the development of sensitization.

Male Sprague-Dawley rats were randomly assigned to treatment groups. Rats in acute groups received a single exposure to intraperitoneal saline, cocaine (15mg/kg), VPA, or VPA (50 or 100 mg/kg) + cocaine. Rats in the sensitized groups received the same treatments, once a day for 6 days. This was followed by a 14 day drug-free period, and then a challenge exposure to cocaine (15 mg/kg). The locomotor response to treatment was measured in an open field arena on the first, sixth and challenge days of treatment using an automated tracking system (Noldus Ethovision XT). All rats were sacrificed 24 hours after the final cocaine administration. Discrete mesocorticolimbic brain regions including the nucleus accumbens, the striatum, and the prefrontal cortex were dissected out and q-rt PCR was performed to measure changes in the cocaine responsive genes c-fos, CART and EGR1.

Repeated administration of cocaine induced a sensitized locomotor response in rats. Locomotion was significantly higher on the sixth day of cocaine exposure than on the first day, and this effect persisted on the challenge day of treatment. Rats that received the HDAC inhibitor VPA prior to each cocaine exposure did not exhibit a significant difference in locomotor behaviour across testing. The results indicate that inhibiting histone deacetylation attenuates behavioural sensitization to cocaine. The effect of VPA on cocaine-responsive genes was also assessed. The findings suggest that chromatin remodeling through histone acetylation plays a significant role in the development of addiction, as well as the propensity to relapse after many years of abstinence, and this mechanism may be a possible target for the prevention and treatment of psychostimulant addiction.
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P-07
Briana Goad

The effect of social separation on behavioral despair, anxiety, learning and memory in the 5XFAD mouse model of Alzheimer's Disease

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Loneliness in humans has been linked to an increased risk for depression and Alzheimer's disease (Wilson et al, 2010, Arch Gen Psychiatry, 64, 234-240). We have previously demonstrated that loneliness can be induced in mice through social separation (Martin & Brown, 2010, Behav Brain Res, 207, 196-207). In this experiment, we examined the effects of social separation on the 5XFAD (B6SJL-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/J) mouse model of AD. Transgenic 5XFAD mice and their wildtype littermate controls were either individually housed at five months of age or remained in group housing with 2-3 littermates. When tested at six months of age, 5XFAD mice had a significantly higher "wildness" score than wildtype mice [$F(1, 26) = 6.26$, $p<0.05$], and separated mice had a higher "wildness" score than group housed mice [$F(1, 26) = 19.12$, $p<0.001$]. In the tail suspension test of depression, wildtype mice spent significantly more time immobile than 5XFAD mice [$F(1, 26) = 16.51$, $p<0.01$], and socially isolated mice spent significantly more time immobile than group housed mice [$F(1, 26)= 4.76$, $p<0.05$]. Wildtype mice took significantly longer to become immobile than 5XFAD mice in the tail suspension test [$F(1, 26)= 7.24$, $p<0.05$]. Furthermore, 5XFAD mice had significantly more bouts of immobility than wildtype mice [$F(1, 26)= 11.54$, $p<0.01$], and separated mice had significantly more bouts of immobility than group housed mice [$F(1, 26)= 4.67$, $p<0.05$]. In the forced swim test, no differences were found between grouped housed or separated mice, or between 5XFAD and wildtype mice. In the light dark box, a test of anxiety, the separated 5XFAD mice had significantly fewer headpokes than the group housed 5XFAD mice [$F(1, 26)=4.91$, $p<0.05$]. 5XFAD mice also had fewer transitions than wildtype mice [$F(1, 26)=6.76$, $p=0.052$]. In tests of learning and memory (Morris Water Maze and Trace Fear Conditioning), there were no differences due to genotype or housing condition. These results indicate that social separation elevated measures of despair in the tail suspension test but not in the forced swim test. Social separation did not effect transgenic and wildtype mice differently, except for the number of headpokes in the light dark box. These results replicate our previous findings on effects

of separation in mice and suggest that there is no indication that the 5XFAD Alzheimer model mice are more sensitive to separation than the wildtype mouse.

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P-08
Joseph Gyekis

Influence of parent of origin on pre-weaning offspring mortality in reciprocal crosses of F1 C57BL/6J and DBA/2J hybrid mice

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C57BL/6J dams give birth to more offspring and have lower offspring survival than DBA/2J dams, especially in their first litters. Reciprocal crosses allow for testing of parent of origin effects on these outcomes. We hypothesized that maternal line transmission of parenting behaviors might lead to higher pup survival in the F1 daughters of D2 mice (D2B6F1 females) than daughters of B6 mice (B6D2F1 females). Alternatively, genomic imprinting could lead to parent-specific genotypic effects. Published rodent studies suggest behavioral transmission of maternal care, thus we hypothesized that F1 dams might exhibit offspring mortality traits similar to their maternal strains. We analyzed data from the breeding of over 1600 F2 mice for a genetic study on B6 and D2 hybrids. All four combinations of F1 strains were mated, and the number of pups at birth and weaning were recorded. D2B6F1 dams tended to have slightly higher numbers of pups at birth than B6D2F1 dams ($t = 1.80$, $p = 0.073$). D2B6F1 dams also had higher pre-weaning pup mortality in their litters than the B6D2F1 dams ($t = 4.26$, $p < 0.001$), and this effect was not explained by the increased litter size. Thus, reciprocal crosses of F1 C57BL/6J and DBA/2J hybrid dams differ in the pre-weaning pup mortality of their litters, and the differences are not consistent with behavioral transmission by the mother, consistent with the possibility of epigenetic effects.
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P-09
Freesia Huang

Methylphenidate improves the behavioral and cognitive deficits of neurogranin knockout mice: A model for attention deficit hyperactivity disorder

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Neurogranin (Ng) is a brain-specific PKC substrate protein, expressed highly in hippocampus and other forebrain areas important for cognitive functions. Deletion of Ng gene in mouse causes deficits in learning and memory, and long-term potentiation (LTP). Environmental enrichment (EE) alone failed to improve the cognitive function of these mice. In children, some cases of learning disability and behavioral abnormality have been attributed to the terminal deletion of a copy of chromosome 11q that includes Ng gene (Jacobsen Syndrome). Further characterization of the Ng knockout mice (KO) revealed that these mutant mice also exhibited hyperactivity and inattentiveness. In the present studies, attempt was made to treat these mice with methylphenidate (MPH), a drug commonly used to treat attention deficit hyperactivity disorder (ADHD), while also maintaining under EE condition. MPH (10 mg/kg /day, i.p.) was given to both wild type (WT) and KO mice for 3 weeks before behavioral testing, while the control WT and KO groups were injected with saline and both injections were continued throughout the testing period. Comparing to the control mice, MPH appeared to reduce the hyperactivity of the NgKO mice as indicated by a decrease in the movement time, and the total and marginal distances traveled in the open field, but an increase in the immobility time in the forced swim chamber. The cognitive memories of MPH-treated mice were also improved as evidenced by a reduction of the latency time to locate the hidden platform in the water maze, and an increase in the freezing time in the contextual fear conditioning. Measurement of the high frequency stimulation (1s x 100 Hz)-mediated LTP also showed a positive effect of MPH on the KO mice. However, the drug only has a marginal effect on the performance of the WT mice and the present treatment regimen could not completely reverse the deficits of NgKO mice. These results suggest that these NgKO mice, based on their hyperactivity and inattentiveness as well as the memory deficiency, can very well be used as an animal model of ADHD for future drug treatment study of this disorder.

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P-10
Ahmed Hussin

The triple test for measuring anxiety in mice: sensitivity to Chlordiazepoxide

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A new test for anxiety, the triple test, combines the open field (OF), elevated-plus maze (EPM) and the light-dark box (LDB) (Ramos, 2008, Trends in Pharmacol Sci 29: 493-498). We investigated the validity of the triple test for mice using the anxiolytic drug Chlordiazepoxide (CDZ). We also investigated the occurrence of one-trial tolerance to CDZ by conducting a second test 24hrs after the first. On two consecutive days, a total of 64 Swiss-Webster mice were injected with different doses of CDZ (0.0 (saline), 1.0, 7.5 and 15.0 mg/kg) and their behaviour was observed for 15 min in the triple test. On day 1, increasing doses of CDZ steadily increased open arm exploration (% time in open arms), entries, time in the light box of the LDB and overall locomotion (distance travelled). On day 2, all groups showed significantly diminished open arm exploration and increased time in the closed arms. This reduction on day 2 is attributed to one-trial tolerance to the drug effects since there was no decrease in general locomotion on day 2 (total distance travelled). The triple test has been now validated with different anxiolytics (such as diazepam, alprazolam and chlordiazepoxide) and different mouse strains (BALB/cJ, DBA/2J, and CD-1) (Fraser et al. 2010, Psychopharmacology, In press). The triple test, which occupies a relatively small area (165 x 65 cm) and has a relatively short trial (15-min), provides both locomotion- and anxiety-related measures; is capable of capturing genetic differences in behaviour; and is sensitive to at least three anxiolytic drugs. Further investgations are currently underway to test for sensitivity to anxiety-inducing drugs.

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P-11
Helen Kamens

Association of the CHRNB2 gene and DSM-IV alcohol abuse and dependence criteria

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Alcohol and nicotine dependence are commonly comorbid behaviors. Data from both human studies and animal models has provided evidence that common genes underlie the response to these two drugs. Recently, varenicline, the α4β2 nicotinic acetylcholine receptor partial agonist that is approved as a smoking cessation aid, has been shown to decrease alcohol consumption in a human laboratory experiment and in animal studies. In this preliminary study we examined the genetic association between variation in the gene that codes for the β2 subunit of the nicotinic acetylcholine receptor subunit (CHRNB2) and DSM-IV alcohol abuse and alcohol dependence symptoms. Nine hundred and sixty-seven caucasian, non-hispanic subjects of probands, siblings and their parents were collected as part of a longitudinal study of adolescent and early adulthood antisocial drug dependence at the University of Colorado. Ten SNPs spanning the CHRNB2 gene were genotyped and analyzed using FBAT-PC^2 and the 11 DSM-IV alcohol abuse and dependence symptoms were included in the analysis. Preliminary results provided suggestive evidence that one SNP in CHRNB2 (rs3811450, $p = 0.05$) was associated with the composite alcohol phenotype. Follow up analysis revealed that this SNP was most strongly associated with the alcohol dependence symptom “important social, occupational or recreational activities given up or reduced because of alcohol consumption.” These data provide suggestive evidence that the β2 subunit of the nicotinic acetylcholine receptor might be important for mediating alcohol abuse and dependence in humans.

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P-12
David Kapfhamer

Protein phosphotase 2A and glycogen synthase kinase 3 beta signalling modulate prepulse inhibition of the acoustic startle response by altering cortical M-type potassium channel activity

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There is considerable interest in the regulation of sensorimotor gating, since deficits in this process could play a critical role in the symptoms of schizophrenia and other psychiatric disorders. Sensorimotor gating is often studied in humans and rodents using the prepulse inhibition of the acoustic startle response (PPI) model, in which an acoustic prepulse gates behavioral output to a startle-inducing stimulus. However, the molecular and neural mechanisms underlying PPI are poorly understood. Here, we show that genetic and pharmacological manipulations of protein phosphatase 2A (PP2A) and glycogen synthase kinase 3 beta (GSK3B) signaling affect PPI. Mice heterozygous for a hypomorphic allele of Ppp2r5d, encoding a regulatory subunit of PP2A, show attenuated PPI. Reduction of PPP2R5D increases the level of (Ser9)phospho-GSK3B, a catalytically inactive form, indicating that PPP2R5D is a positive regulator of GSK3B activity in the brain. Reduction of GSK3 function, in Gsk3B(tm1JW)/+ mice or following acute systemic administration of the GSK3 inhibitor, SB216763, attenuates PPI. Genetic disruption of Kcnq2, encoding an M-type potassium channel protein and putative GSK3B substrate, confers reduced PPI, as does pharmacological inhibition of M-channels systemically with linopirdine. Both SB216763 and linopirdine reduce PPI when directly infused into the medial prefrontal cortex (mPFC). Finally, we show by whole-cell electrophysiological recordings of mPFC neurons that pharmacological inhibition of GSK3 alters firing properties in a manner similar to pharmacological inhibition of M-channels. These data support a previously uncharacterized mechanism by which PP2A/GSK3B signaling regulates M-type potassium channel activity in the mPFC to modulate sensorimotor gating.

P-13
Sulev Kõks

Wfs1 deficient mice have altered response to the morphine and decreased release of striatal dopamine

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Wfs1 deficient mice display reduced motor activity in the open-field test. In addition, we have described reduced sensitivity to the amphetamine in mutant animals compared to the wild-type mice. As wfs1 gene is highly expressed in the mesolimbic structures, the aim of present study was to analyse the efficiency of the morphine actions in the wfs1 deficient mice. We measured motor activity of mice after the administration of different doses (0.5, 1, 2.5, 5, 10, 20 mg/kg) of morphine, we performed GTP binding autoradiography after in vitro stimulation with mu-specific opioid agonist DAMGO ([D-Ala2, N-MePhe4, Gly-ol]-enkephalin) and finally, we evaluated dopamine (DA) release in the striatum of mice with microdialysis. Morphine administration significantly increased the total travelled distance in wild-type mice. The response of mutant mice to the morphine was weaker and in case of 10 mg/kg the difference was statistically significant (genotype effect $F(2, 132)=7.5$, $p<0.01$). As a next step we tested if there is any genotype related neurochemical differences in the action of morphine. We performed in vitro DAMGO induced GTP binding autoradiography in order to evaluate the activation of the m-opioid receptors. In wfs1 mutant mice, DAMGO induced significantly stronger activation of G-protein in the Substantia Nigra of mutant mice ($t=2.213$, $p<0.05$). Microdialysis also indicated some significant differences related to the genotype of animals. The concentrations of DA of the baseline samples were 0.8 nM to 1.4 nm for the wild type animals and 0.6 to 1.3 nM for the Wfs1 KO mice. Repeated measures ANOVA revealed a significant time ($F(1,11)= 18.9$, $p<0.001$) and genotype ($F(1,11)= 14.6$, $p<0.01$) effect, as well as an interaction between these factors ($F(1,11)= 10.6$, $p<0.01$). 10 mg/kg morphine injection increased the striatal DA output in all tested wild type animals (up to 160% from baseline) while in the KO animals only a minor increase (up to 105% from baseline, with a large within group variation) was found. Time-point-by-time-point post hoc analysis (20 - 120 min) considered this difference as statistically insignificant. The application of the [K+]-rich modified Ringer solution increased the striatal DA output in wild type animals (up to 3000%) but the increase of the striatal DA output of Wfs1 KO mice was less pronounced yielding only up to 450% from the baseline values. There was a statistically significant difference between

these two mice groups ($p<0.01$ and $p<0.001$ for the time points 160 and 180 min, respectively). Our results show that the Wfs1 KO mice have reduced behavioral sensitivity to opioid peptides and this finding correlates with neurochemical changes. More detailed analysis of the mechanisms behind these findings needs to be done in further experiments.

P-14
Pyung-Lim Han

Adenylyl cyclase-5 controls the duration of ethanol-induced hypnosis in mice by regulating the mGluR-CaMKIIalpha pathway in the dorsal striatum

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Ethanol produces relaxation and euphoria and at high doses it causes a sleep-like state called hypnosis. Emerging evidence suggests that ethanol exerts its acute inhibitory effects through the modulation of metabotropic glutamate receptor (mGluR) system, but underlying mechanism is not clearly elaborated. Adenylyl cyclase-5 (AC5) is preferentially expressed in the striatum and prefrontal cortex, the brain areas important for motor function and emotion and express mGluRs at high levels.
We investigated whether adenylyl cyclase-5 (AC5) is a key player and mediates mGluRs function in the regulation of ethanol-induced behavior using AC5 knockout (AC5-/-) mice. AC5-/- mice consumed more ethanol and were resistant to ethanol-induced hypnosis, compared to wild-type mice. AC5-/- mice showed a complete loss of the group II mGluR activity. As a result, AC5-/- mice had up-regulated expression of activated CaMKIIalpha in the dorsal striatum, which resulted from overactivation of group I mGluRs caused by failure of antagonistic suppression by group II mGluRs. Consistently, CaMKIIalpha-haplodeficient mice showed enhanced ethanol-induced hypnosis, and stereotaxic infusion of the CaMKIIalpha inhibitor, KN-62, or siRNA-CaMKIIalpha within the dorsal striatum, but not the nucleus accumbens, was sufficient to prolong ethanol-induced hypnosis, supporting a necessary role for CaMKIIalpha in ethanol-induced hypnosis. Together, these results suggest that the AC5-regulated mGluR-CaMKIIalpha pathway in the dorsal striatum is a necessary neural system for the regulation of ethanol-induced hypnosis.
The results of the present study identify AC5-regulated glutaminergic system as a molecular mechanism and the dorsal striatum as a critical brain area for ethanol-induced hypnotic behavior. Within the dorsal striatum, CaMKIIalpha functions as a cellular determinant regulating the duration of ethanol-induced hypnosis. Our results suggest further that the dorsal striatum is a critical site for regulating ethanol-induced hypnotic behavior.
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P-15
Lauren Lederle

Early life developmental influences on fear extinction in inbred mouse strains

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An inability to effectively extinguish fear memories is associated with anxiety disorders such as Phobias and Posttraumatic Stress Disorder. There is growing evidence from rodent studies that the mechanisms underlying fear extinction undergo marked changes over early life development. For example, recent work has shown that fear extinction training may produce permanent fear memory erasure in young (P16-18), but not older (P23+), rats and C57BL/6J (B6) mice (Richardson et al 2007, Gogolla et al 2009). We have recently identified an inbred mouse strain, 129S1/SvImJ (S1), with markedly impaired Pavlovian fear extinction (as compared to C57BL/6J (B6) in adulthood. Here, we asked two major questions. First, does extinction training at P16 produce permanent fear erasure in the S1 strain as it does in the B6 strain? Second, can impaired fear extinction in adulthood be rescued in S1 mice by cross-fostering with B6 mice during various stages of prenatal and postnatal development? To address the first question, S1 and B6 mice underwent Pavlovian fear conditioning at either P16 or P23, and then extinction training and testing on subsequent days. To address the second question, S1 mice were cross-fostered with B6 or other S1 mice, and B6 mice were cross-fostered to S1 mice or other B6 mice at one of three developmental stages: 1) post-weaning (P21), 2) postnatal (P1), or 3) prenatal (E1). Preliminary results indicate that S1 mice conditioned and extinguished at P16-18 showed significantly lesser (but not fully abolished) fear during extinction retrieval and (context) renewal than S1 mice conditioned and extinguished at P23-25. Results obtained thus far also demonstrate that post-weaning cross-fostering with B6 mice was not sufficient to rescue impaired fear extinction in S1 mice. Elucidating the developmental time course of fear extinction in S1 mice, and clarifying the sensitivity of the impaired S1 extinction phenotype to early life influences could shed light on genetic and environmental influences on childhood risk for anxiety disorders.

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P-16
Melanie Leussis

Behavioral and neurobiological characterization of Ank3, a bipolar disorder risk gene

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Bipolar disorder (BP) is a severe mental illness affecting more than 2.5% of the population. Although BP is a highly heritable disease of the brain, the underlying neurobiology is poorly understood. Recent genome-wide association studies have identified ankyrin 3 (ANK3) as a highly significant risk factor for BP. ANK3 encodes the scaffold protein ankyrin G, whose neural functions include localizing ion channels and GABAergic synapses at the axon initial segment, neuronal polarity, and synaptic function. To elucidate the role of ankyrin G in BP, we assessed the behavioral profile induced by Ank3 reduction in mouse brain. Lentiviral-mediated RNA interference was used to knock down Ank3 expression in hippocampus or nucleus accumbens of adult C57BL/6J mice. Mice were assessed for a range of affective behaviors, intermediate phenotypes of BP, and behaviors mediated by the targeted brain regions, as well as phenotype modulation following sub-chronic mood stabilizer treatment. Further, immunohistochemistry was used to examine the impact of chronic mood stabilizer treatment on ankyrin G expression in the brain. Ank3 knockdown in the hippocampus or nucleus accumbens resulted in similar phenotypes that recapitulated some of the clinical symptoms of BP and were partially reversed by lithium treatment. Furthermore, chronic mood stabilizer treatment significantly altered ankyrin G expression at the neuronal AIS in the hippocampus. These results provide insight into the function of ankyrin G in mediating behavior and its modulation by mood stabilizer treatment. Future manipulations of ankyrin G in mice, including creation of genetically accurate knock-in and humanized mouse models carrying the BP risk variant(s), will provide additional in vivo models for testing novel BP therapeutics and for neurobiological studies to delineate ankyrin G's role in BP etiology. Funded by the Stanley Medical Research Institute.

P-17
David Linsenbardt

Bi-directional selection for sensitization to the locomotor stimulant effects of ethanol

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Behavioral sensitization to the acute psychomotor (locomotor) stimulant effects of alcohol (ethanol) is thought to be a heritable risk factor for the development of alcoholism. Evidence from inbred, recombinant inbred, and selectively bred mouse strains, has demonstrated that the acute stimulant response to ethanol is strongly genetically mediated. However, very little is known about the genetic influences involved in the sensitization of this stimulant response. The goal of this work was to determine the heritability of ethanol-induced locomotor sensitization using short-term behavioral selection. C57BL/6J (B6) x DBA/2J (D2) F2 mice were generated from B6D2F1 progenitors, tested for the expression of locomotor sensitization using a standard 14 day ethanol sensitization paradigm (Phillips et al., 1995), and bred for high (HLS) and low (LLS) locomotor sensitization. Sensitization was defined as the difference in ethanol induced locomotion between the 1st and final ethanol challenge day (day14 - day 3 = “sensitization”). Heritability (h2) following 1 generation of selection (S1) was 0.09 and 0.61 for the HLS and LLS lines respectively. Thus, 60% of the difference (decrease) in ethanol-induced locomotor sensitization between the founder population and the LLS line was attributable to genetic differences, whereas genetic differences accounted for only 9% of the difference (increase) in the HLS line. Analysis of the total distance traveled in 15 minutes revealed that the LLS animals displayed significantly lower levels of acute (day 3) and final (day 14) ethanol-induced locomotion compared to HLS animals. Therefore, the difference between lines was not due to ceiling or floor effects since the opposite effect would be observed if this were the case. Timecourse analysis of the difference between the first and final ethanol challenge days revealed that alterations within the first two 5 minute bins (10 minutes) of the testing session contributed the greatest to the between line differences. This is potentially interesting because the F2 founder population displayed stimulation within the 1st 5 minute bin and sedation in the 2nd 5 minute bin compared to baseline activity. Therefore, the differences between the lines may involve both sensitization to the stimulant effects of ethanol and tolerance to its sedative actions. Future work will evaluate the effects of additional selection pressure on this and other ethanol-

related behavioral characteristics, such as voluntary oral ethanol consumption, to determine the nature of the relationship if any exist.
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P-18
Kathryn Martin

Genetics as a "boundary condition" for fear memory reconsolidation

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Impaired fear extinction is characteristic of various neuropsychiatric disorders, including Phobias and Posttraumatic Stress Disorder. Fear memories enter a labile state after reactivation - a process termed reconsolidation. A reconsolidation-like process has been hypothesized to explain the recent finding that reactivating fear memories prior to extinction training facilitates long-term extinction in rats (Monfils et al 2009) and normal humans (Schiller et al 2010). However, whether this reconsolidation-like process can facilitate fear extinction in clinical populations or animal models of impaired extinction has not yet been tested. Indeed, it is known that fear memory reconsolidation in rats is prevented by certain experimental manipulations (so-called "boundary conditions"), such as strong conditioning and hippocampal lesions (Wang et al 2009). We have recently identified an inbred mouse strain, 129S1/SvImJ (S1), with markedly impaired Pavlovian fear extinction (as compared to C57BL/6J (B6), and associated functional abnormalities in a prefrontal-amygdala circuit mediating extinction. Here we tested whether fear memory reactivation prior to extinction training produced improvements and rescue of long-term extinction in B6 and S1, respectively. A conditioned fear memory in B6 and S1 was reactivated by a single exposure to the conditioned stimulus (CS) 60 min prior to 30xCS extinction training. Separate groups of mice were then tested for improvements in long-term fear extinction in 3 experimental conditions that typically cause a reemergence of fear: 1) spontaneous recovery (the fear response to the CS is tested 4 weeks after extinction training), 2) reinstatement (the fear response to the CS is tested after additional unsignaled exposure to the footshock unconditioned stimulus), and 3) renewal (the fear response to the CS is tested in the conditioning context). In a separate experiment, we tested whether S1 (and B6) met a classic test for memory reconsolidation - sensitivity to protein synthesis inhibition - by injecting anicomycin bilaterally into the basolateral amygdala after fear memory reactivation and then testing for short-term and long-term memory fear retrieval. Preliminary results show that memory reactivation prior to extinction training reduced fear during a renewal, but not recovery, test in B6. By contrast, reactivation increased fear during renewal in S1. These data confirm recent

findings in rats and humans that by showing that reactivation facilitates fear extinction in B6 under at least certain test conditions. Reactivation may, however, paradoxically worsen fear in the S1 model of impaired extinction - although additional experiments are needed to confirm this. This work could provide insight into the utility of reactivation as a therapeutic intervention for anxiety disorders, and determine whether genetic factors may impose a boundary condition on fear memory reconsolidation. Research supported by the NIAAA IRP.

P-19
Timothy O'Leary

Impaired motor ability influences learning and memory performance in the aged 5XFAD mouse model of Alzheimer's disease

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The 5XFAD mouse is a novel double transgenic model of Alzheimer's disease (AD), which has the human amyloid precursor protein gene (APP695) with three familial mutations, and the presenilin-1 gene with two mutations. The 5XFAD mouse shows AD-related neuropathology as early as 2-4 months of age (Oakley et al., 2006, J. Neurosci, 26, 10129-10140) and learning and memory impairments at 4-6 months of age (Ohno et al., 2006 Eur. J. Neurosci, 23, 251-260). In this study, we assessed motor ability in the 5XFAD mouse with the rota-rod (6 trials/day for 5 days), locomotor activity and anxiety with the open-field (one 5 min trial), and visuo-spatial learning and memory performance on the Morris water maze (MWM). Mice were tested at 6-9 months of age, where memory deficits have been previously found, and at 12-15 months of age, where memory deficits are expected to be more pronounced. 5XFAD mice did not differ from wild-type mice on the rotarod at 6-9 months of age. At 12-15 months of age, however, wild-type mice took longer to fall from the rotarod than 5XFAD mice, indicating that 5XFAD mice have impaired motor ability at this age. In the open-field, wild-type mice did not differ from 5XFAD in distance travelled at 6-9 months of age, but wild-type mice travelled farther than 5XFAD mice at 12-15 months of age. These differences in activity reflect impaired motor ability in the 5XFAD mice. No genotype differences were found in center square entries in the open-field at 6-9 months of age, but at 12-15 months there was a trend for wild-type mice to make more center square entries than 5XFAD mice, and male mice made more center entries than females. At 6-9 months of age wild-type mice learned to locate the escape platform in the MWM faster than 5XFAD mice. The genotype difference in platform latency occurred because wild-type mice swam faster than 5XFAD mice, as there was no difference in distance travelled. No differences were found between 5XFAD and wild-type mice in percent time spent in the correct quadrant during the memory probe trial at 6-9 months of age. At 12-15 months of age, wild-type mice learned to locate the escape platform faster than 5XFAD mice, and there were no differences in distance travelled. Wild-type mice also swam faster than 5XFAD mice. No differences were

found in probe trial memory performance. These results suggest that by 12-15 months of age 5XFAD mice have impaired motor ability. This impairment confounds latency measures of learning on the MWM, as 5XFAD mice swim slower than wild-type mice. Future research should take this motor impairment into account and use behavioural tests in which motor performance is not confounded with learning ability when testing 5XFAD mice.

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P-20
Shkelzen Shabani

Sensitivity to the anxiogenic effects of methamphetamine reduces vulnerability to methamphetamine consumption

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Methamphetamine (MA) has negative effects (e.g., effects leading to avoidance, anxiogenic effects) in addition to positive rewarding effects (e.g., euphoria, increased attention). The strength of either positive or negative effects may be a determining factor in whether an individual continues to use MA and develops an addiction. This study specifically explored the role of differences in sensitivity to the anxiogenic effects of MA in selectively bred lines of mice that differ in level of voluntary MA consumption. When a 40 mg of MA/L of water solution was offered versus plain water, the first selection generation MA High Drinking (MAHDR) line mice consumed about 4.5 mg/kg of MA, whereas the MA Low Drinking (MALDR) line mice consumed about 1 mg/kg of MA, in an 18-h free access period. In the current study, experimentally naive first selection generation MADR mice were tested for 5 min on the zero maze, beginning 25 min after injection with saline or 3 different doses of MA (1, 2, and 4 mg/kg). Variables scored from recorded behavior were time spent in the open sections of the zero maze, number of entries into the closed and open sections and head dips. In a planned comparison examining the data for differences between non-drug treated MAHDR and MALDR mice (saline group), no significant differences were found, suggesting that the lines do not differ in basal levels of anxiety-like behavior. The entire data set was then analyzed by two-way ANOVA with line and dose as grouping variables. Significant main effects of both line and dose for all three measures were found, but there was no interaction of these variables. In the absence of differences between the saline-treated groups, these results suggest that the line difference was associated with MA exposure. These effects could not be explained by differences in MA-induced activation, since there was no line difference when the same mice were later tested for locomotor response to MA in automated activity chambers. Thus, mice with genes that confer high levels of consumption of MA showed significantly lower sensitivity to the anxiogenic properties of MA, compared to mice with genes that confer lower voluntary consumption of MA. In summary, heightened sensitivity to the anxiogenic effects of

MA plays an important protective role in vulnerability to MA consumption and could play a role in the development of MA addiction.

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P-21
Kurt Stover

Visual and non-visual learning and memory performance in the APPswe/PS1dE9 mouse model of Alzheimer's Disease

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The APPswe/PS1dE9 mouse model of Alzheimer's disease develops amyloid beta plaques in the retina and begins to show retinal degeneration by 12 months of age (Perez et al. 2009, Invest Ophthalmol Vis Sci, 50:793-800). As mice are commonly tested for learning and memory deficits using visually dependent tasks, we studied the relationship between visual ability, learning, and memory in transgenic and wildtype control mice at 6-8 (young) and 20-25 (old) months of age. In the visual water box, we found that young mice performed better than old mice on visual detection ($F(1,41)=23.44$, $p<.0001$) and pattern discrimination ($F(1,41)=31.15$, $p<.0001$), but there was no effect of genotype. Young mice had better visual acuity than old mice ($F(1,41) = 9.43$, $p<.01$) and transgenic mice tended to have worse visual acuity than wildtype mice ($F(1,41)=3.26$, $p = .078$). In the Morris water maze (MWM), a visually dependent test of learning and memory, old mice took longer than young mice to reach the platform ($F(1,41)=44.35$, $p<.0001$) and transgenic mice tended to take longer to reach the platform than wildtype mice ($F(1,41)=3.8$, $p=.0583$). Old mice took longer to reach the visual platform ($F(1,39)=27.49$,$p<.0001$), had slower swim speed ($F(1,40)=43.20$,$p<.0001$), and spent less time in the correct quadrant during the probe trial ($F(1,40)=14.64$,$p=.0004$), than young mice. There was no effect of genotype on these measures. Wildtype mice tended to cross the annulus more frequently then transgenic mice during the probe trial ($F(1,40)=3.99$, $p=.0526$) and young mice crossed the annulus more often than old mice ($F(1,40)=8.67$, $p <.01$). In the conditioned odour preference task, all mice spent more time digging in the CS+ than the CS- odour ($F(1,20)=28.84$, $p<.0005$), and there was no effect of age or genotype on amount of liquid consumed. On the conditioned taste aversion test there was no effect of age or genotype. These results demonstrate an effect of age and genotype on visual acuity and on performance (latency) in the MWM, a vision-dependent task. There was, however, no effect of genotype or age on memory in the non-visual tasks. It appears that amyloid plaques in the retina effect visual acuity and memory performance in the MWM, but not in non-visually dependant tasks. This indicates that there is a confound between sensory and

cognitive deficits during aging and that non-visual tests should be used to ensure that the learning and memory deficits in aging are due to decrements in cognitive rather than sensory function. We are now examining the eyes and brains of these mice for amyloid plaques. Supported by grants from NSERC of Canada and the Alzheimer Association.

P-22
Dekel Taliaz

Hippocampal BDNF knockdown in young rats increases corticosterone level and induces depressive-like behavior

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Exposure to chronic mild stress (CMS) is known to induce anhedonia in adult animals, and is associated with induction of depression in humans. Previously we have shown that CMS does not induce anhedonia in young rats. In addition, we have shown that a knockdown of brain derived neurotrophic factor (BDNF) within the dorsal dentate gyrus (dDG; the hippocampal main input) of adult rats induces anhedonia and depressive-like behavior. Furthermore, while CMS decreased levels of BDNF in the hippocampus of adult animals, it did not decrease BDNF levels in young animals. We have therefore investigated whether the behavioral resilience of young rats to CMS results from their ability to preserve normal hippocampal BDNF levels. In addition, we investigated whether the anhedonic effect of CMS in adult rats depends on the reduced hippocampal BDNF expression. To examine these issues, we sought to reduce BDNF levels in the dDG of young rats (21 days), and measure the consequential effects on behavior with and without exposure to CMS. Additionally, we over-expressed BDNF within the dDG of adult rata and tested the behavioral effects of exposure to CMS. Finally, because the hippocampus has an important role in the negative feedback of the hypothalamus-pituitary-adrenal (HPA) axis and the HPA axis is thought to be altered by stress and depression, we have also measured whether corticosterone secretion is altered by BDNF knockdown or over-expression in the hippocampus in control or CMS-treated rats. While CMS induced anhedonia and depressive-like behavior in young rats with reduced hippocampal BDNF expression, no such behavioral effects of CMS were observed in young control rats. In addition, BDNF knockdown per se (in young rats that were not exposed to CMS) was sufficient to induce anhedonia and depressive-like behavior, but the behavioral effect of knockdown was significantly higher when combined with CMS. Additionally, hippocampal BDNF over-expression in adult rats attenuated the behavioral effects of CMS. Finally, reduced hippocampal BDNF expression caused a significant elevation of corticosterone baseline levels in the serum of young, but not adult, rats. Furthermore, BDNF knockdown caused a significant increase of corticosterone secretion after acute stress in young rats. Taken

together, we show that the behavioral resilience of young rats to CMS depends on BDNF expression in the dDG of the hippocampus and that elevation of BDNF expression in the dDG of adult rats induces behavioral resilience to CMS. Finally, we show that BDNF in the dDG has a significant role at the negative feedback of the hippocampus on the HPA axis.

P-23
Keizo Takao

Behavioral profiles of three C57BL/6 substrains
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C57BL/6 inbred strains of mice are widely used in knockout and transgenic research. To evaluate the loss-of-function and gain-of-function effects of the gene of interest, animal behaviors are often examined. However, an issue of C57BL/6 substrains is not always appreciated though behaviors of mice are known to be strongly influenced by genetic background. To investigate the behavioral characteristics of C57BL/6 substrains, we subjected C57BL/6J, C57BL/6N, and C57BL/6C mice to a behavioral test battery. We performed both a regular-scale analysis whose experimental conditions were tightly controlled and meta-analysis from large number of behavioral data, derived from 700~2,200 mice in total, that we have collected so far through the comprehensive behavioral test battery. Significant differences were found in various behavioral tests, including the open field, rotarod, elevated plus maze, startle response/prepulse inhibition, Porsolt forced swim, and spatial working memory version of the 8-arm radial maze among the substrains. Our results show divergence of behavioral performance in C57BL/6 substrains and therefore indicate that small genetic differences might have great influence on behavioral phenotypes. Thus, genetic background of different substrains should be carefully chosen, equated, and considered in the interpretation of the mutant behavioral phenotypes.
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P-24
Aimee Wong

Timoptic-XE prevents retinal, neural and behavioural signs of visual impairment in the DBA/2J mouse model of glaucoma

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The DBA/2J mouse is used as a model of human pigmentary glaucoma because it shows age-related increases in intraocular pressure (IOP), retinal ganglion cell (RGC) death and visual impairment (Moon 2005, Cell Tis Res, 320, 51; Wong & Brown 2007, Neurobiol Aging, 28, 1577). We evaluated the effect of Timoptic-XE, a conventional glaucoma medication used in humans, on behaviour, IOP, retinal ganglion cell death and transneural labelling in aging DBA/2J mice. Mice were given Timoptic-XE (0, 0.25 or 0.5%) eye drops daily from 2.2 - 12 months of age. At 3, 6, 9 and 12 months of age, mice were tested in a visual detection, pattern discrimination and visual acuity task and their learning and memory ability was evaluated using the Morris water maze and a conditioned odor preference task. IOP of all mice was measured after each battery of behavioural tests. At each age, a subgroup of mice from each drug group were given intraocular injections of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) to visualize and quantify the strength of the connections from the retina to the superior colliculus and a subset of tissue sections were Nissl stained to evaluate somatal size and cell count in the superior colliculus. Retinal flat mounts from these mice were Nissl stained to evaluate retinal ganglion cell number. At all ages tested, mice treated with Timoptic-XE (0.25 and 0.5%) maintained a high level of performance in all behavioural tasks, while 12 month old control mice (0%) exhibited impaired performance in visually-dependent, but not non-visual tasks. Mice treated with Timoptic-XE also maintained significantly lower intraocular pressure from 6 - 12 months of age and had a significantly higher retinal ganglion cell count than mice treated with 0.0% Timoptic-XE at 12 months of age. Behavioural assessments were correlated with IOP, RGC loss, transneural labeling and cell count in the superior colliculus. These results further validate the usefulness of the DBA/2J mouse model of pigmentary glaucoma as these mice respond to the same treatment as humans. Furthermore, this study provides evidence for the “sensory impairment hypothesis” of aging by showing that DBA/2J mice with improved vision can learn visuo-spatial tasks, demonstrating that their impairment is sensory rather than

cognitive and that repairing the sensory deficit facilitates improvement in cognitive function.

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P-25
Jonathan Zombeck

The influence of genes on the locomotor activating effects of cocaine in adolescent versus adult mice

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In C57BL/6J male mice, we and others have observed that adolescents (age 30 days) are significantly less sensitive to the locomotor activating effects of cocaine than adults (age 70 days), i.e., rightward shift in the dose response curve. In all our experiments, animals are tested in their home cage using continuous video tracking. Our results are consistent with many but not all studies using rats and other mouse strains. The reason for this variability is not clear. Important environmental factors include differences in prior handling, doses chosen, and testing conditions (specifically whether in the home cage or novel test chamber) between studies. Genetic differences between strains and species are also likely to account for some of the variability. Inbred mouse strains are useful for partitioning genetic and environmental contributions to a phenotype and have been used to characterize genetic differences in locomotor responses to cocaine and other psychostimulants in adult animals. However, few inbred stains have been tested for age differences in locomotor stimulation from cocaine. The goal of the current study is to examine both sexes from four different inbred stains, C57BL/6J, DBA/2J, FVB/NJ, BALB/cByJ, chosen because each is on a separate branch in the phylogeny of inbred strains, and listed as priority in the Mouse Phenome Database. We hypothesize that adolescents in both sexes and all strains will display decreased sensitivity to the locomotor stimulating effects of cocaine (15 and 30 mg/kg) as compared to adults, but that the magnitude of this age difference will strongly depend on genotype. Results of this and future studies could help identify the origin of basic biological differences between adolescents and adults in behavioral responses to drugs of abuse.

P-26
Greer Kirshenbaum

Mutations in the alpha3 Na+,K+-ATPase underlie a mouse model of bipolar disorder

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The nonexistence of a valid animal model for Bipolar disorder (BD) has been hindering the discovery of the underlying neurobiology of the disease and the development of novel treatments. By ENU mutagenesis, my lab has created a mouse called Myshkin that has a point mutation in the alpha-3 sodium potassium ATPase pump (ATP1A3) causing haploinsufficiency. Myshkin is a compelling new mouse model of BD as it demonstrates construct validity, face validity and predictive validity. The model has construct validity as the underlying pathology of BD may involve altered Na+/K+ ATPase activity. Several studies show that tissues from BD patients have altered ion regulation, consistent with decreased function of the ATP1A3; BD tissues have elevated resting and stimulated intracellular Na+ and Ca+ as well as decreased Na+/K+ ATPase concentrations and increased Na+/K+ ATPase inhibitory ligand concentrations. Similarly, cultured cortical cells from Myshkin show a higher intracellular rise in Ca2+ and slower Ca2+ clearance than wild type cells after glutamate stimulation. Further, mutations in ATP1A1, ATP1A2 and ATP1A3 genes have been identified in BD populations. Myshkin demonstrate many symptoms of BD found in humans. Myshkin show a manic phenotype, demonstrating face validity. Bipolar patients show increased activity in the manic state and Myshkin are hyperactive in the open field, light/dark box and elevated plus maze. Bipolar patients in the manic state engage in risk taking behaviour and Myshkin also show increased risk taking behaviour in the elevated plus maze and light/dark box. Consistent with mania in BD, Myshkin have significantly altered circadian rhythms. Myshkin show increased preference for sucrose indicating hyperhedonia in the sucrose preference test that is also consistent with mania. Sodium valproate and lithium carbonate are common treatments for BD in humans and alleviate many symptoms of the disease. The manic symptoms in Myshkin were attenuated by both sodium valproate and lithium carbonate indicating predictive validity of the model. There is evidence that both humans with BD and Myshkin have decreased function of the ATP1A3; Myshkin show similar symptoms as humans with BD and respond to the most effective drugs for BD. Increasing the activity of the ATP1A3 may be a useful target for

new drug therapies for BD.

P-27
Pamela Arstikaitis

Expression of proteins that promote synapse maturation, but not filopodia production, lead to more stable axo-dendritic contacts
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Dendritic filopodia are dynamic protrusions that are thought to play an active role in synaptogenesis and serve as precursors to spine synapses. However, this hypothesis is largely based on a temporal correlation between filopodia formation and synaptogenesis. We investigate the role of filopodia in synapse formation by contrasting the roles of molecules that affect filopodia elaboration and motility, versus those that impact synapse induction and maturation. Expression of filopodia inducing motifs (FIMs), such as palmitoylated protein motifs found in GAP-43, enhanced filopodia number and motility, but reduced the probability of forming a stable axon-dendrite contact. Conversely, expression of neuroligin-1 (NLG-1), a synapse inducing cell adhesion molecule, resulted in a decrease in filopodia motility, but an increase in the number of stable axonal contacts. Moreover, siRNA knockdown of NLG-1, reduced the number of filopodia and the number of presynaptic contacts formed. Postsynaptic scaffolding proteins such as Shank1b, a protein that induces the maturation of spiny synapses, reduced filopodia number, but increased the rate at which filopodia transformed into spines by stabilization of the initial contact with axons. Taken together, these results suggest that high levels of filopodia elaboration and motility may not necessarily be a rate-limiting step for synapse formation, and suggest that availability of postsynaptic scaffolding proteins are key regulators of synapse production.
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Travel Awardees

Graduate Students
Peter Clark (University of Illinois-Urbana, USA)
Todd Darlington (University of Colorado-Boulder, USA)
Leanne Fraser (Dalhousie University, Canada)
William Giardino (Oregon Health & Science University, USA)
Joe Gyekis (The Pennsylvania State University, USA)
Ahmed Hussin (Dalhousie University, Canada)
David Linsenbardt (Indiana University-Purdue University, USA)
Viara Mileva-Seitz (University of Toronto-Mississauga, Canada)
Tim O'Leary (Dalhousie University, Canada)
Kurt Stover (Dalhousie University, Canada)
Dekel Taliaz (Weizmann Institute of Science, Israel)
Aimee Wong (Dalhousie University, Canada)
Jonathan Zombeck (University of Illinois-Urbana, USA)

Postdoctoral Fellows
Laurence Coutellier (NIMH, USA)
Amy Eisener-Dorman (University of North Carolina-Chapel Hill, USA)
Helen Kamens (University of Colorado-Boulder, USA)
Melanie Leussis (Harvard University, USA)
Megan Mulligan (University of Tennessee-Memphis, USA)
Angela Ozburn (University of Texas-Dallas, USA)
Shkelzen Shabani (Oregon Health & Science University, USA)

Junior Faculty
Igor Ponomarev (University of Texas-Austin, USA)
Justin Rhodes (University of Illinois-Urbana)

Outstanding Young Scientist Awards
Viara Mileva-Seitz (University of Toronto-Mississauga, Canada)
William Giardino (Oregon Health & Science University, USA)
Megan Mulligan (University of Tennessee-Memphis, USA)
Laurence Coutellier (NIMH, USA)

Useful Information

Public Transportation/Metro Transit
www.halifax.ca/metrotransit
One-way fare is $2.25 for buses and ferry service. Buses # 1, 10, 14 stop at Dalhousie University. Buses leave from the stop next to the Student Union Building, Coburg Road or South Street.

Taxi Numbers:
Aerocab: 902-445-3333
Casino Taxi: 902-429-6666 or 902-425-6666
Yellow Cab: 902-420-0000

Meeting Address:
Marion McCain Arts & Social Sciences Building
6135 University Avenue
Dalhousie University Campus
Halifax, Nova Scotia
B3H 1T5

General Halifax Information:

Weather: Halifax in May is generally between 10 and 15 degrees Celsius.

There are three main restaurant/shopping districts. Quinpool Road between Oxford and Robie Streets, Spring Garden Road between South Park and Barrington Streets, and downtown from Brunswick (at the Citadel) to Lower Water Streets (towards Harbourfront). The proximate edges of these are within walking distance of campus.

Hours of operation in the downtown area are generally 0900 to 1830; some are open until 2100. On Sundays, businesses are open from 1200 to 1800.

Take time to walk along the Harbourfront! Halifax is generally a safe city to travel in, but as always, in crowds, be aware of your personal possessions.

Map of Downtown Halifax