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INTERNATIONAL BEHAVIOURAL AND NEURAL GENETICS SOCIETY

Genes, Brain & Behaviour

Abstract Book

13th Annual Meeting

Rome, Italy

May 10-14, 2011

Editing, graphic design and cover by Cosimo Marino Curianò

Genes, Brain and Behaviour 2011

13th Annual meeting of the International Behavioural and Neural Genetics Society

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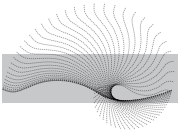

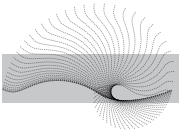

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Laura Ricceri
Igor Branchi

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Contents

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E-mail: info@globushotel.com
Website: www.globushotel.com
Price: 10-15 euros/hours
The hotel is just around the corner from the meeting site.

Contact local Meeting Organizer Maria Luis Scattoni
E-mail marialuisa.scattoni@iss.it
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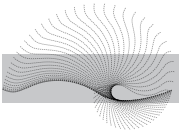


Meeting Programme

Tuesday 10th May 2011

THE LONG WAY FROM GENOTYPE TO BEHAVIORAL PHENOTYPE: POTENTIAL PITFALLS AND COPING STRATEGIES

Organizers:
Laura Ricceri, Walter Adriani and Igor Branchi
Dept of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy

Morning session: ***Managing variability***
Chairs: *Laura Ricceri and Walter Adriani*

10.30 **From mouse to zebrafish in behaviour genetics: new promises and new challenges**
Robert Gerlai
Dept of Psychology, University of Toronto Mississauga, Ontario, Canada

11.00 **Mouse phenotyping: dealing with genetic background and environment as sources of noise and bias**
David P. Wolfer
Institute of Anatomy, University of Zürich, Zürich, Switzerland

11.30 *Coffee break*

12.00 **Towards a common denominator in a complex array of phenotypes: the SERT knockout rat**
Judith Homberg
Dept of Cognitive Neuroscience, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

12.30 **A checklist for documenting laboratory factors affecting the behavioral phenotyping of mice**
Richard E. Brown
Psychology Dept and Neuroscience Institute, Dalhousie University, Halifax, Canada

13.00 *Lunch*

Afternoon session: ***Focusing on the context***

Chair: *Igor Branchi*

14.00 **How does sex count? Implications of sex differences for behavioral phenotyping**
Paola Palanza
Dept of Evolutionary and Functional Biology University of Parma, Parma, Italy

14.30 **Genetic and environmental modulators of developmental trajectories**
Francesca Cirulli
Dept of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy

15.00 *Coffee break*

15.30 The relevance of the context: naturalistic vs. laboratory settings
Hans Peter Lipp
Institute of Anatomy, University of Zürich, Zürich, Switzerland

Closing session: **Perspectives in behavioral genetics: lessons from pioneers**
Enrico Alleva and Hans Peter Lipp

16.00 New perspectives in behavioral genetics: back to the future
Douglas Wahlsten
Department of Psychology, University of North Carolina, Greensboro, NC, USA

16.30 Back to the future: peering under the carpet of early behavior genetics
Giorgio Bignami,
Dept of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy.

17:30 IBANGS 2011 Meeting Welcome Reception

IBANGS 2011 MEETING

Wednesday 11th May 2011 (Aula Pocchiari)

8:30-9:30 **Keynote Lecture**
Development of the cerebral cortex and cognitive disorders
Zoltán Molnár

9:30-10:00 *Coffee Break*

10:00-12:00 **Symposium 1: "Genetics of Behavioral Plasticity in Model Systems"**
Chair: *Catharine H. Rankin*

Social environment influences performance in a cognitive task in natural variants of the foraging gene
Nancy R Kohn

Social transmission of oviposition site preference in Drosophila melanogaster
Marine Battesti

A Drosophila model for alcohol reward
Karla Kaun

Pain circuits in worms
William Schafer

High Throughput Behavioral Characterization of Habituation of a Mutant Library of Nervous-system-biased Strains of Caenorhabditis elegans
Catharine H. Rankin

12:00-13:00 *Lunch*

13:00-15:15 **Selected Talks Session (1)**
Chairs: *Maria Luisa Scattoni and Joshua Dubnau*

Impulsivity as an endophenotype for neurodevelopmental disorders
Paul Sabandal

Csnk1e is a genetic regulator of sensitivity to psychostimulants and opioids
Camron D Bryant

A mutation in circadian gene Clock (Δ19) increases the vulnerability for cocaine addiction in mice
Erin B Larson

The effects of early social enrichment on adult resilience to depression-like phenotype are associated with BDNF epigenetic modifications
Igor Branchi

Imaging genetics of FOXP2 in dyslexia
Holger Kirsten

Modulation of Rho GTPases by the bacterial protein CNF1 improves the behavioural phenotype and reverses astrocytic atrophy in a mouse model of Rett syndrome
Bianca De Filippis

15:15-15:45 *Coffee Break*

15:45-17:15 ***IBANGS General Business Meeting***

Thursday 12th May 2011 (Aula Pocchiari)

8:30-9:30 **Distinguished Scientist Award Lecture**

Mouse Models of Autism to Test Hypotheses about Causes and to Discover Effective Treatments
Jacqueline N. Crawley

9:30-10:00 *Coffee Break*

10:00-12:00 **IBRO SPONSORED SYMPOSIUM:** ***Social learning of one-other's emotional state in mice and people: neural and neurochemical mediation.***

Chairs: *Hee-Sup Shin, Aron Weller*

Neural mechanisms underlying empathy behaviors in mammals
Hee-Sup Shin

Social Learning of Fear to Natural Threats: An Oxytocin – Estrogen Interplay
Martin Kavaliers

Estrogenic involvement in social recognition and social learning
Elena Choleris

Sex differences in the effects of oxytocin on social cognition
Simone G. Shamay-Tsoory

12:00-13:00 *Lunch*

13:00-15:00 ***Symposium 2: Genetic origins of alcohol and nicotine dependence.***
Chair: *Mary-Anne Enoch*

TTC12-ANKK1-DRD2 and CHRNA5-CHRNA3-CHRNB4 Influence Different Pathways Leading to Smoking Behavior from Adolescence to Mid-Adulthood.
Francesca Ducci

The role of CHRNA4 and CHRNB2 in Alcohol and Nicotine Behaviors
Helen M Kamens

Convergent Evidence Implicates CRHBP in Stress-Related Disorders Including Alcoholism
Mary-Anne Enoch

Genetic influences on alcoholism risk: an examination of human laboratory phenotypes of stress, craving, and subjective intoxication.
Lara A. Ray

15:00-15:30 *Coffee Break*

15:30-16:30 ***Outstanding Travel Awardees***
Chairs: *Mark Rutledge-Gorman and Jacqueline N. Crawley*

Junior Faculty Travel Award:
Francesca Ducci

Postdoctoral Fellow Travel Award:
Karla R Kaun

Graduate Student Travel Award:

Genome-wide association study to identify variants associated with amphetamine sensitivity in humans
Amy B Hart

Maternal Separation is associated with an altered response to stress and epigenetic alterations
Rachel L Kember

16:30-18:30 **Poster Session and Wine Tasting** (Aula Marotta)

Friday 13th May 2011 (Aula Pocchiari)

8:30-9:30 **Young Scientist Award Lecture**

Subunit selectivity of GABAA receptor modulation of limited-access alcohol intake in a mouse model.
Stephen L. Boehm II

9:30-10:00 *Coffee Break*

10:00-12:00 **Symposium 3:** ***Understanding social behaviour: genetic, neural and pharmacological approaches.***
Chairs: *Cathy Fernandes, Viviana Trezza*

Social behavior and social communication in mice: unraveling alterations in autism animal models
Maria Luisa Scattoni

Neural pathways involved in social play behavior in adolescent rats
Viviana Trezza

Modelling the effects of cannabis on social behaviour using adolescent mice: hunting for genetic and environmental risk factors for schizophrenia"
Cathy Fernandes

Dissecting molecular machines and neural circuits coordinating a simple aggregation behavior
Mario De Bono

12:00-13:00 *Lunch*

13:00-15:00 **Selected Talks Session (2)**
Chairs: *Kyung-An Han and Richard Brown*

An integrative approach to studying gene-behavior associations in the human brain
Turhan Canli

Modeling Pathology by Lentiviral Manipulation in Accumbal DAT, and therapy by a Novel 5-HT(7) Agonist Drug
Walter Adriani

A genetic pathway to memory also induces changes in the genome sequence in neurons during aging
Lisa Prazak

The p66Shc gene shortens lifespan in laboratory mice but promotes survival in mice exposed to food competition and winter temperatures in the wild
Alessandra Berry

Profiling trait anxiety: Transcriptome analysis reveals cathepsin B (Ctsb) as a novel candidate gene for emotionality in mice
Ludwig Czibere

The role of Glyoxalase 1 in anxiety-like behavior
Margaret G. Distler

15:00-15:30 *Coffee Break*

15:30-17:30 **Symposium 4: *Zebrafish, a novel model organism in neurobehavioural genetics.***
Chair: *R Gerlai*

Dopamine modulates Akt signaling and alters GABAergic neuron development and motor behaviour in zebrafish larvae
Vincent Tropepe

Visual Behavior Testing in Larval and Adult Zebrafish
Stephan C.F. Neuhauss

A behavioural function for adult neurogenesis in zebrafish?
William HJ Norton

Using zebrafish to identify developmental and genetic mechanisms contributing to drug dependence and psychiatric disease.
Caroline H Brennan

Genetic and neurobiological mechanisms of alcohol in the brain: zebrafish a novel research tool.
Robert Gerlai

19:00-21:30 **Social Banquet**

Saturday 14th May 2011 (Aula Pocchiari**)**

8:30-9:30 **Keynote Lecture**

How to find quantitative trait genes in the mouse
Jonathan Flint

9:30-10:00 *Coffee Break*

10:00-12:00 **Symposium 5:** ***Novel Insights into CaMKII Function in Learning & Memory from Mouse Genetic Studies.***
Chair: *K. Peter Giese*

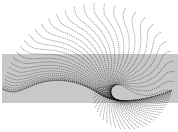
ßCaMKII plays a non-enzymatic role in hippocampal synaptic plasticity and learning by targeting aCaMKII to synapses
Ype Elgersma

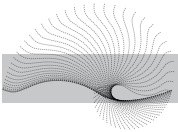
The role of kinase activity of Ca2+/calmodulin-dependent protein kinase II in hippocampus- and amygdala-dependent memory
Yoko Yamagata

Manipulation of Real-time Memory Traces by inducible protein knockout in the mouse brain
Joe Z. Tsien

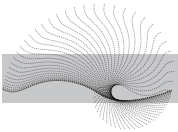
Identifying alphaCaMKII autophosphorylation-dependent and –independent mechanisms of memory formation
K. Peter Giese

ßCaMKII, "immature dentate gyrus" and memory
Tsuyoshi Miyakawa





Abstracts



Wednesday 11th May 2011

Wednesday 11th May 2011

8:30 – 9:30 Keynote Lecture

Development of the cerebral cortex and cognitive disorders

Zoltán Molnár MD DPhil
Department of Physiology, Anatomy and Genetics

Akil et al (Science, 327:1580, 2010) have identified the integration of genomics and neural circuits as the future of psychiatric research. "Integrating the tools of genomics and neural science is needed to reveal causes of psychiatric illness and to suggest new strategies for treatment and prevention." Psychiatric illnesses such a schizophrenia, autism and mood disorders appear to arise from abnormal development of neural circuits. Gross anatomical malformations and their associated mental deficits usually become evident in the first few years of life, if not before. But subtle changes, such as slight changes in neuronal positioning or the ratios of different cell types or the strength of their connections are less easy to identify and they are likely to contribute to, if not cause, diverse forms of mental illness. Understanding the interactions between the susceptibility genes and the environmental influences in these diseases is the current challenge of developmental neurobiology. Neurogenesis, neuronal migration and differentiation are conducted with an unfolding genetic program that can be altered or interrupted by environmantal influences. Building the brain is like a house of cards. The early connections provide the foundation of the adult structure, and disruption of these may be the source of many developmental flaws. Cerebral cortical developmental disorders (including schizophrenia and autism) and perinatal injuries involve cortical neurons with early connectivity, and the major hindrance of progress in understanding the early neural circuits during cortical development and disease was a lack of reliable markers for specific cell populations. Due to the advance of powerful approaches in transcriptome analysis and the utility of models with reporter gene expressions in specific cortical cell types our knowledge of the early cortical circuits is rapidly increasing. This field benefited from recent developments in mouse genetics in generating models with subtype specific gene expression patterns, powerful cell dissection and separation methods combined with detailed expression analysis. With focus on the rodent cerebral cortex I shall illustrate the progress made in the understanding of the formation of the early intracortical and extracortical circuitry during normal and altered cortical development.

Supported by MRC, BBSRC and EU FP7

10:00 – 12:00 Symposium 1:
Genetics of Behavioral Plasticity in Model Systems
Chair: Catharine Rankin

Social environment influences performance in a cognitive task in natural variants of the foraging gene

NR. Kohn[1], C.J. Reaume[2], J.G. Burns[2], M.B. Sokolowski[2], F. Mery[1]
[1]*Laboratoire Evolution, Génome et Spéciation, CNRS, Bâtiment 13, Gif sur Yvette Cedex, France,* [2]*Department of Biology, University of Toronto at Mississauga, Missauga, Canada*

How social environment affects behaviour has recently received increased attention. In Drosophila, natural genetic variation in the foraging gene, which encodes for a cGMP-dependent protein kinase (PKG), affects the foraging activity of larval and adult flies. Sitters (fors) tend to be more sedentary and aggregate within food patches whereas rovers (forR) have greater movement within and between patches of food. Additionally, forR and fors vary in their performance on a number of cognitive tasks. We hypothesized that these variants would also differ, in a classical olfactory conditioning test, depending on whether they were in groups or alone. Individual performance was affected by PKG activity. In fors, but not in forR, the acquisition of information was facilitated by the social interaction (being in a group). In forR, but not in fors, the type of social interaction (being with other forR or with other fors) affected learning and memory. Also, naïve individual forR tended to follow groups of conditioned fors but not groups of conditioned forR. Our results suggest that for mediates some social aspects involved in learning and memory in Drosophila melanogaster. Also, the traditional way of studying ol factory learning with groups may not be a good indicator of individual performance.
Funding support: European Research Council under the European Community's Seventh Frame-work Programme (FP7/2007–2013)/ERC Grant agreement no 209540 to FM

Social transmission of oviposition site preference in Drosophila melanogaster

M. Battesti[1,2], D. Joly[1], F. Mery[1]

[1]*Lab Evolution Genome and Speciation, CNRS Gif sur Yvette;* [2]*University of Paris-Sud, Orsay, France.*

The studying of social learning has always been a crucial issue in order to understand the emergence and maintenance of new behaviors. In the present study we show for the first time social transmission of oviposition preference in a non-social insect, Drosophila melanogaster, using a "transmission chain" approach. Such studies simulate natural traditions by training a population of founders to perform a task, and gradually replacing these experienced individuals (demonstrators) with naive animals (observers). This will allow to test whether the original behavior remains in the population in spite of this change in personal. Using this protocol, we can investigate whether a preference for a specific oviposition site can be transmitted in an environment where observers can not only observe demonstrators but also interact with them and other observers. Such situation also permits direct interactions with the environment and acquisition of personal information. This type of experiments allows appreciating how individuals weight social information compared to personal information. Results open new perspectives on the study of the evolution of sociality, its impact on behavioral evolution and its genetic bases.

A Drosophila model for alcohol reward

K.R. Kaun[1], R. Azanchi[1], Z. Maung[1], J. Hirsh[2], U. Heberlein[1]
[1]*Department of Anatomy, University of California, San Francisco, CA 94143-2822, USA;* [2]*Department of Biology, University of Virginia, Charlottesville, VA 22904, USA*

The rewarding properties of drugs contribute to the development of abuse and addiction. However, the mechanisms underlying the hedonic properties of abused drugs are poorly understood. We have developed a new paradigm to investigate the motivational properties of ethanol in the genetically tractable model, Drosophila melanogaster. We found that flies learn to associate cues with ethanol intoxication and, although transiently aversive, the experience leads to a long-lasting attraction for the ethanol-paired cue, implying that intoxication is rewarding. We also found that like mammals, flies require activation of dopaminergic systems to express attraction for ethanol. However, we found that dopamine was required specifically for retrieval, rather than acquisition of memories of ethanol reward. Moreover, we found that flies acquire, consolidate, and retrieve these rewarding memories using distinct and sequential sets of neurons of the mushroom body. Finally, we conducted an unbiased genetic screen and found that mutations in scabrous, encoding a fibrinogen-related peptide that regulates Notch signaling, disrupt the formation of memories for ethanol reward. Our results establish Drosophila as an attractive organism to study the molecular genetic and neural mechanisms underling the hedonic properties of ethanol.

Pain circuits in worms

M. Chatzigeorgiou, M. Ezcurra, Buyun Zhao, William Schafer
MRC Laboratory of Molecular Biology, Cambridge UK

Elucidating the mechanisms by which nervous systems process information and generate behaviour is among the fundamental problems of biology. Ultimately, it is desirable to understand these processes at the most basic level, that of molecules and cells. We are investigating these questions using the nematode Caenorhabditis elegans, which has an anatomically simple and well-defined nervous system and is tractable to molecular and classical genetic analysis. Using molecular genetics and in vivo optical neuroimaging, we are investigating how the activities of individual neurons correlate with behaviour, and how genes with interesting behavioural phenotypes affect the activities of individual neurons in defined neural circuits.

We are particularly interested in sensory circuits that mediate responses to noxious stimuli. C. elegans contain polymodal neurons that, like mammalian pain-sensing nociceptors, respond to aversive mechanical, chemical, and thremal stimuli. The responses of these neurons are modulated by monoamines as well as neuropeptide signaling. We have used these neurons to investigate molecular mechanisms involved in the sensation of harsh touch, extreme heat and cold. In addition, we have identified interneurons that integrate convergent sensory information and allow nociceptive and mechanosensory neurons to modify each others response properties. We anticipate that these studies will reveal basic conserved principles of sensory transduction and neural circuit function.

High Throughput Behavioral Characterization of Habituation of a Mutant Library of Nervous-system-biased Strains of Caenorhabditis elegans

C.H. Rankin[1], A.C. Giles[1], T. Timbers[1], E. Ardiel[1], N.A. Swierczek[2], R.A. Kerr[2]
[1]*University of British Columbia, Vancouver, BC, Canada,* [2]*Janelia Farm Research Campus - HHMI, Ashburn, VA, USA,* *Funding support NSERC Discovery Grant to CHR, an NSERC post-graduate scholarship to ACG, HHMI JFRC Visitor Program funding to CHR and ACG, HHMI JFRC Funding to NAS and RAK.*

Habituation is the most simple and fundamental form of learning and is measured as a decrease in response to a repeated stimulus. We assay habituation of the tap withdrawal response in C. elegans using the Multi-Worm Tracker (MWT). The MWT allows rapid characterization of tap habituation and therefore enables the testing of large numbers of mutants. Typically, we track 60-80 animals at a time on a single Petri plate seeded
thinly with E. coli; four replicates of each manipulation are performed to eliminate variability between Petri plates. We observe spontaneous behavior for 10 minutes prior to the stimulation protocol to assay locomotion and spontaneous reversal rates. Thirty mechanical taps to the side of the plate are then administered at a 10 second inter-stimulus interval. In wild-type worms, the tap initially elicits a reversal response that gradually habituates with repeated presentations. We have collected and tested a nervous-system-biased mutant library (~700 strains) by cross-referencing a list of 2073 genes with predicted neural function based on domain structure (Sieburth et al. Nature:436, 2005) with the list of available strains at the Caenorhabditis Genetics Center. We will discuss variation of the behaviors found between the mutant strains; in particular, we have verified the defects in known tap habituation mutants and found new mutants that have relatively wild-type behavior on all measures save the rate/level of habituation of the tap withdrawal response. We have followed up with 30 of these habituation mutants by testing secondary (and for some tertiary) mutations in the gene of interest. The gene that causes the strongest habituation effect when mutated encodes a regulator of G-protein signaling (RGS) known as eat-16. We are currently following up on this gene as well as others to yield new insights into the molecular mechanism of habituation.

13:00 – 15:00 Selected Talks Session (1)

Impulsivity as an endophenotype for neurodevelopmental disorders

Y.C. Kim, P. Sabandal, J. Lim, K.A. Han
Department of Biological Sciences, Border Biomedical Research Center Neuroscience & Metabolic Disorders Project, University of Texas at El Paso, El Paso, TX 79968, USA

Neurodevelopmental disorders such as autism, schizophrenia and attention deficit hyperactivity disorder are associated with impulsive behavior. Understanding of the neurobiological basis of impulsivity may provide important insights into the pathogenesis mechanisms or interventions of associated disorders. To this end, we developed a behavioral assay, No-Go test, to explore Drosophila mutants displaying loss of impulsive control. When subjected to the No-Go test, flies lacking dopamine transporter (DAT) inhibited locomotor activity initially but lost the inhibition and manifested impulsive hyperkinetic activity (movement speed higher than 60 mm/sec). The DAT mutant's impulsive activity was fully suppressed by inactivating D1, but not D2, dopamine receptor. This indicates a crucial role of dopamine and D1 receptor in impulse control. Remarkably, the DAT mutant's impulsive activity is sensitive to visual input of other flies. Thus, this study provides a novel system to unravel the mechanism by which the dopamine system interacts with environmental and social stimuli in impulse control and may help understand the neurobiological basis of impulsivity associated with neurodevelopmental disorders.

Funding support: ABMRF/The Foundation for Alcohol Research, USDA/NIFA 2010-65105-20625, and NIH/RCMI 5G12RR008124 grant

Csnk1e is a genetic regulator of sensitivity to psychostimulants and opioids

C.D. Bryant[1], L. Zhou[3,4], C. Olker[3,4], M.H. Vitaterna[3,4], F.W. Turek[3,4,5], A.A. Palmer[1,2]
[1]Department of Human Genetics, University of Chicago, Chicago, IL USA; [2]Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL USA; [3]Center for Sleep and Circadian Biology, Northwestern University, Evanston, IL USA; [4]Department of Neurobiology and Physiology, Northwestern University, Evanston, IL USA; [5]Department of Psychiatry and Behavioral Sciences, Northwestern University, Evanston, IL USA

Recent evidence suggests that Csnk1e, the gene encoding casein kinase 1-epsilon, modulates sensitivity to amphetamines in mice and humans. Additionally, a CSNK1E genetic variant is associated with heroin addiction, suggesting that it may affect opioid sensitivity as well. In this study, reciprocal congenic lines of C57BL/6J (B6) and DBA/2J (D2) origin capturing Csnk1e were phenotyped for both methamphetamine (MA) and opioid sensitivity. We also tested the phenotypic consequence of a Csnk1e null or tau mutation. B6.D2Csnk1e mice carrying a 4.63 cM introgressed region of D2 origin (78-86.8 Mb) on a B6 background showed an increase in MA sensitivity whereas D2.B6Csnk1e mice carrying a 0.55 cM introgressed region of B6 origin (78.7-81.6 Mb; Csnk1e = 79.2 Mb) on a D2 background showed a decrease. B6.D2Csnk1e also demonstrated an increase in fentanyl sensitivity. Mice harboring a null Csnk1e mutation showed an increase in MA sensitivity whereas mice harboring the tau mutation showed a decrease in MA sensitivity. Last, preliminary results indicate that the new selective Csnk1e inhibitor PF-4800567 affects the locomotor response to MA and fentanyl. The results provide selective genetic and pharmacological evidence that Csnk1e regulates sensitivity to multiple classes of abused drugs.

A mutation in circadian gene Clock (Δ19) increases the vulnerability for cocaine addiction in mice.

E.B. Larson, A. Ozburn, D.W. Self, C.A. McClung
University of Texas Southwestern Medical Center, Department of Psychiatry, Dallas, TX 75390-9070

Chronic exposure to drugs of abuse produces disruptions in circadian rhythms, and these alterations may further exacerbate drug addiction. Disruption of the circadian gene Clock (ClockΔ19) increases cocaine-mediated effects on locomotor activity, conditioned place preference, and brain stimulation reward, suggesting an increased sensitivity to reward in these mice. In order to understand how this altered Clock function may affect addictive behavior, we assessed cocaine self-administration in wild-type (WT) or ClockΔ19 mutant (MUT) mice. We found that both WT and MUT mice showed similar learning and readily acquired food self-administration. In contrast, MUT mice acquired cocaine self-administration (0.5 mg/kg, i.v.) more readily than WT mice, and a greater percentage reached acquisition criteria. Interestingly, the effect of the ClockΔ19 mutation on cocaine self-administration was more pronounced when testing was conducted in the light cycle (ZT2) compared to the dark cycle (ZT14). When withdrawn from cocaine, MUT mice showed greater cocaine-seeking behavior when returned to the drug-taking environmental context, and they showed greater cue-induced reinstatement of extinguished cocaine-seeking behavior. Together, our results indicate that the Clock gene plays an important role in drug reward and reinforcement, and that disruption of Clock function may increase the vulnerability for addiction.

Funding support: NIDA R01-DA023988

The effects of early social enrichment on adult resilience to depression-like phenotype are associated with BDNF epigenetic modifications

I. Branchi[1], S. Santarelli[1], I.D'Andrea[1], N. Karpova[2], E. Castren[2], E. Alleva[1]
[1]*Section of Behavioural Neurosciences, Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, 00161 Rome, ITALY;* [2]*Sigrid Jusélius Laboratory, Neuroscience Center, University of Helsinki, P.O. Box 56, 00014 Helsinki, Finland.*

During the early postnatal period, the brain is highly plastic and environmental factors play a major role in shaping its structure and function. In order to investigate the effects of the early experiences on adult resilience to major depression and, in particular, to elucidate the molecular mechanisms mediating such effects, we exposed mouse pups to an early social enrichment: the Communal Nest (CN). CN consists in a single nest where three mothers keep their pups together and share care-giving behavior until weaning. When compared to mice reared in standard laboratory conditions (SN), adult CN mice showed endophenotypes of improved resilience to depression, such as more elaborate social competences, reduced anhedonia and lower corticosterone levels following social stress. These behavioral and neuroendocrine modifications were accompanied by higher hippocampal BDNF levels. The epigenetic structure of the BDNF gene was found to be modified accordingly, CN mice having a significantly more acetylated BDNF gene – i.e. a more permissive structure for gene expression -- compared to SN mice. Overall, the present findings suggest a role for BDNF epigenetic modifications in mediating the effects of the early social enrichment on adult resilience to depression.

Funding support: 11US/1 and 530F/51, both to EA

Imaging genetics of FOXP2 in dyslexia

A. Wilcke[1,2], C. Ligges[4], J. Burkhardt[1], M. Alexander[5,6], C. Wolf[7], E. Quente[1], P. Ahnert[3], A. Becker[8], B. Müller-Myhsok[7], S. Cichon[5,6,7], J. Boltze[1,2], H. Kirsten[1,2,3,10]

[1]*Translational Centre for Regenerative Medicine (TRM), Leipzig;* [2]*Fraunhofer Institute for Cell Therapy and Immunology (IZI), Leipzig;* [3]*Institute for Medical Informatics, Statistics and Epidemiology (IMISE), Leipzig;* [4]*Department of Child and Adolescent Psychiatry, Friedrich Schiller University, Jena;* [5]*Department of Genomics, Life & Brain Center, University of Bonn, Bonn;* [6]*Institute of Human Genetics, University of Bonn, D-53111 Bonn;* [7]*Max Planck Institute of Psychiatry, RG Statistical Genetics, Munich;* [8]*Department of Neuropathology, University of Bonn Medical Center, Bonn;* [9]*Institute of Neuroscience and Medicine (INM-1), Structural and Functional Organization of the Brain, Genomic Imaging Research Center Juelich, Juelich;* [10]*LIFE Center (Leipzig Interdisciplinary Research Cluster of Genetic Factors, Phenotypes and Environment), Universität Leipzig, Germany*

Dyslexia is a common developmental disorder with a strong genetic basis. However, links between genetic polymorphisms and phenotypic deficits are largely unknown. We investigated a possible role of variants in FOXP2 in dyslexia using imaging genetics. To our knowledge, this study represents the first usage of functional imaging genetics in dyslexia. We initially applied a case/control study (n=245) for prioritisation of FOXP2 polymorphisms for later use in imaging genetics. SNP rs12533005 showed nominally significant association (p=0.025). Therefore, this variant was chosen to study the influence of carriage of the rs12533005-G risk variant on brain activity. In fMRI, the contrast of a rhyming task vs. fixation revealed a significant main effect for the factor "genetic risk" in a temporo-parietal area known for its role in phonological processing as well as a significant interaction effect between the factors "disorder" and "genetic risk" in activation of inferior frontal brain areas. In addition, this variant was shown to alter expression of FOXP2 transcripts in silico as well as in vivo in human hippocampus tissue. Our data support a role of FOXP2 variants in language processing relevant for the development of dyslexia and demonstrate a framework for the application of imaging genetics in dyslexia research.

Funding Support: German Federal Ministry of Education and Research, 0313909, 01KN0702, 01GS08144, Interdisciplinary Center for Clinical Research Jena, T.P. 1.2/B 307-04004) habilitation grant from the Hochschul- und Wissenschaftsprogramm/Thuringia, European Regional Development Fund (ERFD), European Social Fund, Free State of Saxony (LIFE Center, University of Leipzig)

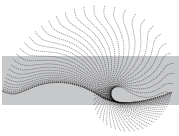
Modulation of Rho GTPases by the bacterial protein CNF1 improves the behavioural phenotype and reverses astrocytic atrophy in a mouse model of Rett syndrome

B. De Filippis[1], A. Fabbri[2], D. Simone[1], R. Canese[1], L. Ricceri[1], F. Malchiodi[1], C. Fiorentini[2*], G. Laviola[1*]

[1]Dept. Cell Biology & Neuroscience, [2]Dept. Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Roma, Italy. [*]Equally senior authors

Rho GTPases are crucial molecules in neuronal plasticity and cognition, as confirmed by their role in non-syndromic mental retardation. Brain Rho GTPases modulation induced by the bacterial protein Cytotoxic Necrotizing Factor 1 (CNF1) in non-mutant mice reshapes the actin cytoskeleton and enhances neurotransmission and synaptic plasticity. We investigated the capacity of CNF1 administration to contrast the neurobehavioural phenotype in a mouse model of Rett syndrome (RTT), a neurodevelopmental disorder and a genetic cause of mental retardation. After CNF1 icv inoculation, fully symptomatic Mecp2-308 male mice reached a wt-like profile of circadian locomotor cyclicity, showed an amelioration of forelimb-related nest building capacity and a better performance in a cognitive fear-conditioning task. Brain sections were immunohistochemically characterized. No changes on markers of dendritic tree and synapses were found. By contrast, astrocytes showed evident signs of atrophy in the corpus callosum and hippocampus of mutant mice, which were dramatically reverted by CNF1 treatment. In CNF1-treated mice, in vivo MRS detected brain changes in metabolites involved in glial integrity and bioenergetics, pointing to improved mitochondrial functionality. Our observations support a novel role for astrocytes in mediating the beneficial effects of CNF1, and point to Rho GTPases as new targets for pharmacological intervention in RTT.

Funding Support: E-RARE-EuroRETT network and Foundation Jerome LeJeune to GL.



Thursday 12th May 2011

Thursday 12th May 2011

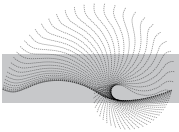
8:30-9:30 Distinguished Scientist Award Lecture

Mouse Models of Autism to Test Hypotheses about Causes and to Discover Effective Treatments

Jacqueline N. Crawley
Laboratory of Behavioral Neuroscience, Intramural Research Program, National Institute of Mental Health, Bethesda, MD

Autism is a neurodevelopmental disorder defined by unusual social interactions, social communication deficits, and repetitive behaviors with restricted interests. Recognition of the high heritability of autism spectrum disorders, up to 90% in monozygotic twins, prompted a worldwide search for genes associated with autism. No single gene has emerged as the common cause of autism spectrum disorders. Instead, multiple candidate genes and chromosomal deletion regions have been identified, each in a few individuals, consistent with the multigenic nature of many neuropsychiatric disorders. Targeted mutations in many of the autism candidate genes have now been generated in mice. Mouse models offer useful translational tools to test hypotheses about single gene mutations, chromosomal loci duplications and deletions, DNA methylation and other epigenetic regulators, neuroanatomical and neurophysiological abnormalities, immune dysfunctions, environmental contaminants, diets, and other proposed causes of autism.

The key to success in phenotyping mouse models of autism spectrum disorders is employing robust, replicable, highly relevant assays. Our laboratory and others are developing mouse behavioral paradigms with conceptual analogies to the three diagnostic symptoms of autism. This presentation will focus on behavioral tests for mice that maximize face validity to the defining symptoms of autism. The core deficit in reciprocal social interactions is modeled longitudinally across developmental stages with social approach and social interaction tasks. Social communication in mice is investigated with measures of the emission, detection, and responses to social olfactory cues and vocalizations. Motor stereotypies, repetitive behaviors, insistence on sameness, and narrow restricted interests are analyzed in mice by quantitating stereotyped motor behaviors, repetitive self-grooming, perseveration during the reversal phase of Morris water maze and T-maze spatial tasks, and restricted



exploration of complex environments. Mouse behavioral assays relevant to the associated symptoms of autism, including anxiety, seizures, sleep disruption, mental retardation, and hyperreactivity to sensory stimuli, may provide further insights into the phenotypes of a mouse model of autism spectrum disorder. Comprehensive control parameters for general health, sensory abilities, and motor functions are routinely scored to avoid overinterpretation of artifacts.

Both forward and reverse mouse genetics are employed in our laboratory to understand the genetic basis of social interaction, communication, and repetitive behaviors. BTBR T+tf/J, an inbred strain that displays traits relevant to all three diagnostic criteria for autism, will be used to illustrate phenotypes of a strong mouse model of autism. Representative results in mice with targeted mutations in candidate genes for autism will be described, including the Shank family of synaptic scaffolding protein genes that mediate synapse development.

Mouse models offer ideal translational systems to discover therapeutic targets and evaluate treatment efficacy. Our mouse models that incorporate the most robust traits with high face validity to the diagnostic symptoms of autism are being applied to the search for effective therapeutics. Early preclinical results will be presented on drug treatments and behavioral interventions that reverse components of autism-relevant behavioral phenotypes in the BTBR mouse model of autism.

10:00-12:00 IBRO SPONSORED SYMPOSIUM:

Social learning of one-other's emotional state in mice and people: neural and neurochemical mediation

Chairs: Hee-Sup Shin, Aron Weller

Neural mechanisms underlying empathy behaviors in mammals

Hee-Sup Shin

Center for Neural Science, Korea Institute of Science and Technology, 39-1 Hawolgok-dong, Seongbuk-ku, Seoul, 136-791, Republic of Korea

Empathy is the capacity to identify with or understand another's situation, feeling, or motivation. Empathy is essential for social behaviors in mammals. Fear can be acquired by observation of others suffering from a fearful situation. Such observational fear is thought to be dependent on empathy. We have developed a behavioral assay to monitor observational fear in the mouse. Mice display freezing behavior upon observing other mice experiencing repetitive foot shocks, and they get conditioned for this context. Demonstrator mice who are closely related to the observer mice elicited a stronger fear response in the observer mice, a phenomenon similar to the situation in empathy behaviors in humans. Using the lesion techniques, we identified the medial pain system of the brain as the important brain substrate necessary for the observational fear; sensory thalamic nuclei appeared unnecessary for the behavior. Recording in vivo of the local field potential revealed an increase of neuronal activities at the theta frequency in the ACC and amygdala during the observational fear behavior. In addition, the theta rhythms were synchronized between the two regions during this learning. Furthermore, we found that Cav1.2 Ca2+ channels in ACC are necessary for the behavior. These results demonstrate the functional involvement of the affective pain system and Cav1.2 channels in the ACC in observational social fear by empathy. Currently, we are trying to define the circuits underlying the cortical mechanism for this social learning behavior, and see a tendency for cortical lateralization in this behavior. I will discuss current findings in the context of the neural circuits for social learning.

Social Learning of Fear to Natural Threats: An Oxytocin – Estrogen Interplay

M. Kavaliers[1], E.Choleris[2]

[1]*Department of Psychology, University of Western Ontario, London, ON, Canada;* [2]*Department of Psychology, University of Guelph, Guelph, ON, Canada*

Social learning of fear and avoidance responses has a rich ecological and ethological background. Investigations with rodents have begun to focus on the social modulation of emotional behavior and responses in the context of the broad behavioral definition of empathy and it's underlying genetic and neural bases. Here we will briefly consider social learning of fear and avoidance to natural threats ("micropredators", biting flies), the social recognition and avoidance of the threat of infection and its modulation by direct and indirect social information, and the involvement of genes for oxytocin and estrogen receptors. Estrogenic regulation of the OT system and their control of social recognition and adaptive social behaviors such as the acquisition of defensive behaviors, the avoidance of parasitized conspecifics, mate choice and mate copying will be described.

Funding support: Natural Sciences Engineering Research Council of Canada, Grant 3341 (MK) and Grant 400212 (EC).

Estrogenic involvement in social recognition and social learning

E. Choleris, A.E. Clipperton-Allen, A. Phan
Department of Psychology, University of Guelph, Guelph, ON, Canada

Estrogens and their receptors (ER) affect behavior either via long-term (hours-days) gene-transcription dependent mechanisms (genomic effects), or via rapid (minutes) cell signaling mechanisms. We studied estrogenic regulation of two aspects of social cognition: recognition of a novel vs a familiar conspecific, and social learning, the acquisition of information from conspecifics.

Using global ERaKO and ERbKO mice and rapid ERa and ERb agonist administrations we showed a regulatory involvement of ERa and a possible modulatory role for ERb in social recognition and dendritic spines. Conversely, acute long-term ERa agonist treatment blocked the social transmission of food preferences, while acute long-term administration of an ERb agonist prolonged the expression of the socially learned food preference. When administered postacquisition (before or after memory consolidation), acute long-term ERa and ERb agonists blocked social learning. This suggests that ERa and ERb impair memory mechanisms involved in social learning. However, ERb may promote social learning via social mechanisms (nature of social interactions).

We find a complex interplay between estrogens and their receptors on social cognition. ERa favors social recognition but blocks social learning, possibly because of different types of memory and brain areas involved, hypothalamus/amygdala for social recognition and hippocampus for social learning.

Funding support: Natural Sciences and Engineering Research Council of Canada, Grant 400212

Sex differences in the effects of oxytocin on social cognition

Simone G. Shamay-Tsoory,
Department of Psychology, Haifa University, Haifa, Israel

Evolutionary psychology has increasingly demonstrated differential sex-related hormonal and behavioral responses to social interactions. Despite the dominant role of the hormone oxytocin (OT) in female reproductive processes, little is known about sex differences in the way that OT affects social behaviors. The current studies employed double-blind, placebo-controlled crossover designs in order to investigate sex differences in the effect of OT on social understanding. In the first experiment, 52 women and men performed the Interpersonal Perception Task (IPT), which requires identification of the relationship between people interacting in video clips according to three categories: kinship, intimacy, and competition, following the administration of either OT or placebo. A three-way interaction between treatment, task category, and sex indicated that OT had a selective effect on improving kinship recognition in women, but not in men, whereas men's performance was improved following OT only for competitive relationships. In the second study, we examined the effects of intranasal administration of OT on emotional and cognitive theory of mind abilities. Our results demonstrate that OT impaired theory of mind accuracy scores in males but not in females. Collectively, the findings suggest that OT involves sex-specific characteristics

13:00-15:00 ***Symposium 2: Genetic origins of alcohol and nicotine dependence.***
Chair: *Mary-Anne Enoch*

TTC12-ANKK1-DRD2 and CHRNA5-CHRNA3-CHRNB4 Influence Different Pathways Leading to Smoking Behavior from Adolescence to Mid-Adulthood.

F. Ducci*, M.D., PhD[1,2]; M.K.* M.Sc[3,4]; AP, MD, PhD[5]; AL Hartikainen, M.D., PhD[6]; J. Veijola, M.D., PhD[7]; M Isohanni, MD, PhD [7]; P Charoen[8,9], MSc, MPhil; L Coin[8], PhD; C Hoggart, PhD[8]; J Ekelund[10], PhD; L Peltonen, MD, PhD[10,11,12]; N Freimer, MD[13,14,15]; P. Elliott, MBBS, PhD, FmedSci[8,16]; G. Schumann* M.D.1; MR Järvelin* MD, PhD[3,4,5,8]

[1]*Institute of Psychiatry, Kings College, London, UK;* [2]*St George's University, London, UK;* [3]*Institute of Health Sciences, University of Oulu, Oulu, Finland;* [4]*Biocenter Oulu, University of Oulu, Oulu, Finland;* [5]*Department of Lifecourse and Services, National Institute of Health and Welfare, Oulu, Finland;* [6]*Institute of Clinical Medicine / Obstetrics and Gynecology, University of Oulu, Oulu, Finland;* [7]*Institute of Clinical Medicine / Psychiatry, University of Oulu, Oulu, Finland;* [8]*School of Public Health, Department of Epidemiology and Biostatistics, Imperial College London, London, UK;* [9]*Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand;* [10]*Institute for Molecular Medicine (FIMM), University of Helsinki, Helsinki, Finland;* [11]*Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK;* [12]*Broad Institute of Harvard and MIT, Cambridge, USA;* [13]*Center for Neurobehavioral Genetics, University of California, Los Angeles, California USA;* [14]*The Jane and Terry Semel Institute for Neuroscience and Human Behavior, Los Angeles, California, USA;* [15] *Department of Psychiatry, University of California, Los Angeles, California, USA;* [16]*MRC-HPA Centre in Environment and Health, London, UK*

Our ability to detect gene effects on complex behaviour is dependent upon the context in which their effects are measured, and it is becoming clear that we cannot ignore that genes act within a complex network that includes other genes, environmental variables, and developmental timing. In this presentation I will speak about the combined effects of two gene clusters, CHRNA5-CHRNA3-CHRNB4 and TTC12-ANKK1-DRD2, and psychosocial factors on smoking behavior during adolescence and mid-adulthood. Participants from this study included 4,762 subjects from a general population based prospective Finnish Cohort. Smoking behavior was collected at age 14 and 31 years(y). Maternal smoking, socio-economic status, and novelty seeking personality trait were also collected. Several SNPs in both gene clusters were significantly associated with smoking. The most significant were in CHRNA3 (rs1051730, P=1.1x10-5) and TTC12 (rs10502172, P=9.1x10-6). The T allele at CHRNA3-rs1051730 was more common among regular smokers than occasional/non-smokers [OR~ 1.28 at both age 14y and 31y]. The C allele at TTC12-rs10502172, was more common among regular/occasional smokers than non-smokers [OR~ 1.33 at 14y and 1.14 at 31y]. TTC12-rs10502172 effect on smoking in adulthood was partially mediated by its effect on smoking in adolescence and by high novelty seeking. Effect of CHRNA3-rs1051730 on smoking in adulthood was direct.

The role of CHRNA4 and CHRNB2 in Alcohol and Nicotine Behaviors

Helen M. Kamens, Robin P. Corley, Nicole R. Hoft, Matthew B. McQueen, Michael C. Stallings, Sandra J. Brown, Thomas J. Crowley, Christian J. Hopfer, John K. Hewitt, Marissa A. Ehringer
University of Colorado, Institute for Behavioral Genetics, Boulder, CO 80301, USA.

Alcohol and nicotine are often co-abused. The $\alpha 4\beta 2$ nicotinic acetylcholine receptors are the most abundant acetylcholine receptors located in the brain. Multiple studies performed in animal models have provided evidence for a role of these receptors in alcohol and nicotine behaviors. A few human genetic studies examining the CHRNA4 and CHRNB2 genes have been conducted, but association results with human alcohol and tobacco behaviors remain mixed. The current study attempts to clarify the role of these genes in two large independent longitudinal samples: the Center on Antisocial Drug Dependence (CADD) and the Genetics of Adolescent Antisocial Drug Dependence (GADD). In the CADD sample, 10 SNPs were selected which capture the majority of the genetic variation in the region and tested for association with DSM-IV alcohol and nicotine abuse and dependence using FBAT. SNP rs7543174 in CHRNB2 showed an association with nicotine dependence ($p<0.005$). We are examining these same SNPs in the GADD sample to determine if this finding can be replicated. Collectively, the animal data and emerging human genetic data provide support for the hypothesis that variation in the genes encoding the $\alpha 4$ and $\beta 2$ subunits may be associated with alcohol and nicotine behaviors.

Supported by K01 AA015336, R01 AA017889 P60 DA011015, R01 DA012845, R01 DA021905, R01 DA021913

Convergent Evidence Implicates CRHBP in Stress-Related Disorders Including Alcoholism

M-A Enoch [1], C.A. Hodgkinson [1], C. Marietta [1], B. Albaugh [1], A. Roy [2], D. Goldman [1]
[1]*Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892, USA;* [2]*Psychiatry Service, Department of Veterans Affairs, New Jersey VA Health Care System, East Orange, NJ, USA.*

The actions of corticotropin releasing hormone (CRH), a key regulator of the neuroendocrine stress response, are moderated by a high-affinity binding protein (CRHBP). We first identified CRHBP at a genome-wide significant linkage peak in a whole genome linkage scan for an EEG intermediate phenotype for alcoholism. Our subsequent analyses have shown that the same distal CRHBP SNPs and haplotypes are associated with stress-related disorders including alcoholism in several, ethnically diverse populations: alcohol use disorders in U.S. Caucasians (N = 188) and Southeastern American Indians (N = 164) and anxiety in Plains Indians (N = 328) and Finnish Caucasians (N = 270). Moreover, CRHBP interacts with childhood trauma both independently and additively with FKBP5, another stress-related gene, to predict suicidal behavior in treatment seeking alcohol /drug dependent African Americans (N = 398). All documented associations in our and other studies have been with SNPs at the 3' end of CRHBP at the location of an alternative isoform that we have identified in brain. In this isoform the terminal exon is spliced out in favor of two alternative 3' exons resulting in a truncation of the protein at the C-terminus that might affect protein folding / stability and binding of CRH. Ongoing functional studies will be presented.

Funding Support: the Intramural Research Program of the National Institute on Alcohol Abuse and Alcoholism, NIH; RO1 DA 10336-02 grant to AR from the National Institute of Drug Abuse, NIH.

Genetic influences on alcoholism risk: an examination of human laboratory phenotypes of stress, craving, and subjective intoxication.

L. A. Ray[1,2]

[1]*University of California Los Angeles, Department of Psychology, Brain Research Institute, and* [2]*Department of Psychiatry and Biobehavioral Sciences*

This presentation will describe two studies employing human laboratory paradigms to identify genetic risk markers for alcoholism. Study #1 examines genetic determinants of stress-induced and cue-induced craving in heavy drinkers by testing single nucleotide polymorphisms (SNPs) of the corticotrophin releasing hormone binding protein (CRH-BP) gene and the mu-opioid receptor (OPRM1) gene. Participants were followed at 6 and 12 months to prospectively examine the predictive utility of genetic and phenotypic markers on alcohol-related outcomes. Study #2 is a placebo-controlled alcohol administration followed by cue-exposure in a sample of patients with alcoholism. Participants were prospectively genotyped for the Asn40Asp SNP of the OPRM1 gene and additional SNPS in the mu, kappa, and delta, opioid receptors were assayed for genetic analysis of subjective intoxication and craving phenotypes. Results of study #1 revealed that a SNP of the CRH-BP gene (rs10055255) moderated stress-induced craving ($p < .05$) and predicted greater levels of subjective tension and negative mood ($p < .05$) following stress-induction. The Asp40 allele of the OPRM1 was associated with greater cue-induced alcohol craving following the neutral imagery condition ($p < .05$). Analyses of study #2 are currently underway. These studies leverage experimental paradigms to elucidate the underlying genetic underpinnings of biobehavioral risk markers for alcoholism.

Funding Support: ABMRF, the Foundation for Alcohol Research

15:30 - 16:30 Outstanding Travel Awardees

Genome-wide association study to identify variants associated with amphetamine sensitivity in humans

A.B. Hart[1], B.E. Engelhardt[2], A. Skol[3], M. Stephens[1,4], H. de Wit[5], A.A. Palmer[1,5]
[1]Department of Human Genetics, University of Chicago, Chicago, IL 60637 USA; [2]Department of Computer Science, University of Chicago, Chicago, IL 60637 USA; [3]Department of Medicine, University of Chicago, Chicago, IL 60637; [4]Department of Statistics, University of Chicago, Chicago, IL 60637 USA; [5]Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL 60637 USA

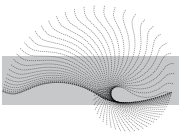
Humans vary in their response to d-amphetamine, and this variation has been shown to have a genetic basis. Previous work has focused on studying polymorphisms in genes that have known functions or that have been previously implicated in related phenotypes. These 'candidate gene' studies are not well suited for identifying novel genes. In an effort to pursue a less biased approach, we undertook a genome-wide association study with a cohort of 382 non-drug-abusing individuals. d-amphetamine was administered over three double-blind sessions of randomized order (placebo, 10 mg, and 20 mg) during which subjects were extensively phenotyped utilizing drug response questionnaires (POMS, DEQ, ARCI) and physiological measures. Subjects were genotyped on the Affymetrix 6.0 array at 906,598 SNPs. Imputation was used to expand coverage to approximately 8 million SNPs. We employed sparse factor analysis to generate ten biologically meaningful factors that were able to explain the bulk of the phenotypic variance. These factors represented drug response measurements as well as non-drug influenced baseline personality and physiological characteristics. Association mapping was performed in a Bayesian framework with the software package BIMBAM. From these analyses, we have already obtained numerous SNP associations with Bayes factor > 10-4, which reflect suggestive evidence for association. Analyses using more traditional frequentist statistics are ongoing. By taking advantage of both high-quality phenotyping and phenotype data, we have identified novel genetic variants associated with both non-drug-influenced measures and amphetamine sensitivity in humans.

Funded by: DA027545 and DA021336

Maternal Separation is associated with an altered response to stress and epigenetic alterations

R.L. Kember, E. Dempster, L.C. Schalkwyk, J. Mill and C. Fernandes
Institute of Psychiatry, Kings College London

Early life stressors are often implicated in the development of psychiatric disorders. Despite this, the factors that mediate the interaction between gene and environment remain largely unknown. Recent studies provide increasing evidence that such effects are mediated by epigenetic processes. Using a maternal separation paradigm, we investigate phenotypic and epigenetic changes following early life stress in two inbred strains of mice, C57BL/6J and DBA/2J. Early life stress induced a number of behavioral changes, many of which were sex and strain dependent. Changes in anxiety behavior, exploration of novel objects, and baseline activity following maternal separation were observed. Additionally, we found an increase in corticosterone after a stressful event in male, C57BL/6J mice that had undergone maternal separation compared to controls. Finally, we examined genome wide DNA methylation levels in the hippocampus across promoter regions, and compare to levels of gene expression in the same tissue. This study contributes to a growing body of recent literature asserting that epigenetic changes are implicated in translating environmental effects into a behavioral phenotype. Importantly, using two inbred mouse strains allows us to acknowledge the genetic contribution towards both behavioral and epigenetic modifications. Studies such as these will increase understanding of the role of environmental, genetic and epigenetic mechanisms in the development of adverse phenotypes.



Friday 13th May 2011

Friday 13th May 2011 (Aula Pocchiari)

8:30 – 9:30 Young Scientist Award Lecture

Subunit selectivity of $GABA_A$ receptor modulation of limited-access alcohol intake in a mouse model.

Stephen L. Boehm II
Department of Psychology, Indiana University-Purdue University Indianapolis, Indianapolis, Indiana.

$GABA_A$ receptors are believed to mediate a number of alcohol's behavioral effects. Functional $GABA_A$ receptors/channels are composed of five protein subunits. However, great diversity exists with respect to subunit composition of the functional receptor (α1-6, β1-3, γ1-3, δ, ε, θ1-3, ρ1-3), with most $GABA_A$ receptors being composed of two α, two β, and one γ subunit. This has led to speculation that alcohol action at $GABA_A$ receptors may depend on which subunits are present or absent, a view that has been supported by studies in mutant mice (Boehm et al., 2004). More recently, work has begun to characterize the subunit selectivity of various drugs that interact with the $GABA_A$ receptor. Ongoing work in my lab takes advantage of some of the more selective drugs to determine the subunit selectivity of alcohol's behavioral actions in mice. In one line of investigation, we have recently demonstrated that Ro 15-4513, a benzodiazepine inverse agonist at the $GABA_A$ receptor, dose-dependently attenuates limited-access alcohol intake when site-specifically microinjected into the posterior ventral tegmental area (VTA) of female C57BL/6J mice (Melòn and Boehm, in press). Ro 15-4513 may have produced these effects via selective actions at δ-subunit containing $GABA_A$ receptors (Olsen et al, 2007). However, Ro 15-4513 may also exhibit selectivity for γ2-containing receptors (Linden et al., 2011). Further complicating this picture is that estrous status appeared to influence the effectiveness of Ro 15-4513 in a subset of the mice tested. Our current efforts are focused on the intra-VTA effects of THIP, another compound believed selective for δ-containing $GABA_A$ receptors (Boehm et al., 2006). Our goal is to more definitively determine 1) whether δ-containing $GABA_A$ receptors in the posterior VTA truly modulate limited-access ethanol intake in mice, and 2) whether estrous status is an important determinant of this modulation. Our efforts to develop an even more selective lentiviral vector-mediated knockdown approach will also be discussed.

10:00-12:00 ***Symposium 3: Understanding social behaviour: genetic, neural and pharmacological approaches.***

Chairs: Cathy Fernandes, Viviana Trezza

Social behavior and social communication in mice: unraveling alterations in autism animal models

Maria Luisa Scattoni
Neurotoxicology and Neuroendocrinology Section, Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy.

Rodents are highly social animals that communicate predominantly in the ultrasonic range of sound frequencies. Ultrasonic vocalizations are emitted by mice under different social conditions throughout their lifespan. Pups separated from the nest emit vocalizations, signals which the parents use to locate the straying pup and retrieve it to the nest. Adult mice of both sexes produce complex ultrasonic vocalization patterns in different experimental/ social contexts such as play, aggressive or sexual interactions. This talk will focus primarily on evaluation of social behavior and ultrasonic vocalizations emitted during dyadic interactions in inbred and outbred mice. Mouse strains emit different pattern of vocalizations in these social contexts associated with different levels of social investigation. In addition, experimental evidence indicates as vocalizations are a valuable tool for identifying alterations in several mouse models of human neurodevelopmental disorders, starting from those in which deficits in social communication are a primary core symptom e.g. autism spectrum disorders.

Supported by Giovani Ricercatori – Ricerca finalizzata 2008 – "Non invasive tools for early detection of Autism Spectrum Disorders", Fascicolo GR3.

Neural pathways involved in social play behavior in adolescent rats

V. Trezza[1,2], L.J.M.J. Vanderschuren[2,3]

[1]*Department of Biology, University "Roma Tre", Rome, Italy;* [2]*Rudolf Magnus Institute of Neuroscience, Department of Neuroscience and Pharmacology, University Medical Center Utrecht, Utrecht, The Netherlands;* [3]*Department of Animals in Science and Society, Division of Behavioural Neuroscience, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.*

Social play behavior, also known as rough-and-tumble play, is the most characteristic and energetic social behavior displayed by young mammals. Social play is critical for the development of behavioral flexibility and the acquisition of social and cognitive competence. Furthermore, social play is a natural reinforcer: it can be used as incentive for place conditioning and maze learning, and it is modulated through neurotransmitter systems implicated in the motivational and pleasurable aspects of natural and drug rewards, such as endogenous opioids and endocannabinoids. Thus, previous studies have shown that opioid receptor agonists enhance social play, whereas opioid receptor antagonists suppress it. Indirect cannabinoid agonists, that increase endocannabinoid signalling by inhibiting endocannabinoid inactivation, enhance social play, through interaction with opioid and dopaminergic neurotransmission. Interestingly, other drugs that act on brain reward mechanisms, such as ethanol and nicotine, also increased social play, acting through opioid, cannabinoid and dopamine receptors. Investigation of the brain regions involved has thus far identified the nucleus accumbens and amygdala as important sites for opioid and cannabinoid modulation of social play. Together, our data indicate that interacting opioid, cannabinoid and dopaminergic systems within the corticolimbic circuits underlying incentive motivation and reward modulate the expression of social play behavior.

Modelling the effects of cannabis on social behaviour using adolescent mice: hunting for genetic and environmental risk factors for schizophrenia

C. Fernandes

[1]*Department of Psychosis Studies, Institute of Psychiatry, King's College London, London, UK.*

Cannabis is one of the most commonly used illicit drugs and is generally considered to be safe. However, cannabis use, especially during adolescence, confers an increased risk of developing psychotic disorders. Cannabis does not have severe long term effects for the majority of users so there must be predisposing factors that increase vulnerability, or resilience, to the effects of cannabis. There is likely to be a genetic contribution but little is known about the mechanisms by which cannabis alters vulnerability to schizophrenia. Abnormalities across a range of behavioural domains are known to occur in schizophrenia, including a broadly defined, but severe social deficit. The endocannabinoid system plays an important role in the development of social behaviour and therefore cannabis use during adolescence could have potentially harmful effects on this key behavioural domain. While human studies are valuable in identifying associations with cannabis use, it is only possible to combine exposure to the main components of cannabis with specific developmental periods using animal models. I will discuss the research using animal models to investigate the effects of cannabis during development on social behaviour and to determine the degree to which susceptibility to the effects of cannabis on behaviour is genetically influenced.

Funded by a Research Councils' UK (RCUK) fellowship

Dissecting molecular machines and neural circuits coordinating a simple aggregation behavior

Mario De Bono
MRC Laboratory of Molecular Biology, Cambridge, UK

The well-defined nervous system of C. elegans offers special opportunities to understand the molecular and neural underpinnings of behaviour. One of the most complex behaviors exhibited by C. elegans is aggregation. Using forward genetics we have identified a series of molecules that modulate this behaviour. These include TRP channels, neuropeptides, cGMP transduction pathways and novel oxygen sensors. Using neuron-specific transgenic rescue experiments we have identified neural circuits in which these molecules act. Neurons regulating aggregation include O2 and CO2 sensors, nociceptors, and neurons that convey information about feeding state. We have functionally linked these neurons to behavioural submotifs underlying aggregation, and using genetically encoded Ca2+ indicators, have identified patterns of neural activity that correlate with the behaviour. Using optogenetics we have tested directly how these identified neurons contribute to behavioural output.

13:00 – 15:00 Selected Talks Session (2)

An integrative approach to studying gene-behavior associations in the human brain

T. Canli[1,2], D. Moser[1], M. Jurkiewicz[2], E. Chen[3,4], E. Hatchwell[3,5], D.A. Bennett[6]
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Studies of gene-behavior associations tend to focus on the link between gene polymorphisms and phenotypes of interest. This approach does not consider the interplay and regulatory complexities of gene transcription and translation, nor does it address tissue-specific gene regulatory processes in the central nervous system. Here, we introduce an integrative approach that begins with postmortem human brain tissue from donors with well-characterized behavioral phenotypes. We characterize this tissue, as a function of phenotype (e.g., high versus low ante mortem trait anxiety), using global protein expression profiling and whole-genome gene expression at the level of mRNA and miRNA. We then focus on genes that are differentially expressed as a function of phenotype at either the mRNA level or at the protein level, and focus on putative miRNAs as regulatory agents between mRNA and proteins. We then investigate the function of these putative miRNAs by cloning the 3'UTR region of genes of interest into a pmirGlo vector and feeding target miRNAs, confirming the results at the mRNA level with qPCR and the protein level with Western blots. Using this approach, we are investigating both well-known candidate genes as well as novel genes significantly associated with anxious phenotypes in our dataset.

Funding Support: National Science Foundation, BCS-0843346, National Institute on Aging, 1 R01 AG034578-01 to T.C., and and National Institute on Aging R01AG15819, R01AG17917 to D.B.

Modeling Pathology by Lentiviral Manipulation in Accumbal DAT, and therapy by a Novel 5-HT(7) Agonist Drug

W. Adriani[1], R. Canese[1], E. Lacivita[2], M. Leopoldo[2], J.L. Dreyer[3], G. Laviola[1]

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Neuro-psychiatric disorders depend on key neurotransmission proteins that are distributed in limbic prefrontal-cortex and striata. Dopamine transporter, DAT, is involved in motivation and inhibitory control; Serotonin "7" receptors, 5-HT(7), are involved in emotions and sleep-wake rhythm. Experiment 1 - We inoculated lentiviral vectors for intra-accumbal modulation of DAT gene in rats: control, constitutive silencing (SIL), regulatable enhancement (DAT+), or both (DAT+SIL). While anxiety was elevated in SIL rats, DAT+SIL and DAT+ rats (to a lesser extent) displayed a strong preference for large/uncertain over small/certain rewards, which disappeared upon switch-off over DAT-enhancer, consistently reappearing afterwards. In-vivo MRI-guided 1H-MRS (at 4.7T) revealed changes within bio-energetic metabolite (phospho- and total-creatine) indicating, for these rats, a functional up-regulation of dorsal striatum (dStr) and/or down-regulation of ventral striatum (NAcc). Experiment 2 - A novel 5-HT(7) agonist, LP-211, was preliminarily assessed in male mice. Acute LP-211 0.25mg/kg increased locomotion and time spent in lit-chamber, but diminished new-environment exploration. We then studied spontaneous circadian rhythms under constant-light: LP-211 0.25mg/kg induced significant phase advances. Conclusion. Altered DAT function in forebrain dopamine is associated with an ADHD-like profile of enhanced gambling-proneness. Agonist modulation over 5-HT(7), beneficial for sleep disturbance and anxiety, might be proposed also for ADHD therapy.

Funding support: "Under 40" Young-Investigator Project (coordinated as PI by W.A.) from Italian Ministry of Health

A genetic pathway to memory also induces changes in the genome sequence in neurons during aging.

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One of the central tenets of modern genetics holds that the genetic material of the germline is faithfully transmitted to offspring and that all somatic cells share the same genetic code. Known exceptions to this include a low rate of somatic mutation during mitosis, and orchestrated recombination to provide diversity in the immune system. We will provide evidence that in Drosophila, a genetic pathway that normally is activated for memory also induces an orchestrated reorganization of the nucleotide sequence of certain chromosomal loci. We will advance a model in which the normal function of this signaling pathway is to enact synaptic plasticity, but over time, a maladaptive consequence is an age-dependent decline. Mutations in this signaling pathway cause defects in memory, and also accelerate the genomic reorganizations that likely lead to cell death. Given the highly conserved nature of the genes involved, and the similarity in their known cellular roles, suggests that this mechanism may be at play across animal phyla, including in humans.

Funding support: NIH and DART Neuroscience Inc.

The p66Shc gene shortens lifespan in laboratory mice but promotes survival in mice exposed to food competition and winter temperatures in the wild

A. Berry[1], M. Giorgio[2],[1] Berniakovich[2], H.P. Lipp[3], E. Alleva[1], P.G. Pelicci[2], F. Cirulli[1]
[1]*Department of Cell Biology and Neurosciences, Division of Behavioural Neuroscience, Istituto Superiore di Sanità, 00161 Roma, Italy;* [2]*Department of Experimental Oncology, European Institute of Oncology, 20139 Milan, Italy;* [3]*Division of Neuroanatomy and Behavior, Institute of Anatomy, University of Zürich, 8057 Zürich, Switzerland*

P66Shc-/- mice are characterised by increased longevity and reduced oxidative stress. P66Shc plays also a role in energetic metabolism promoting fat accumulation, p66Shc-/- mice resulting lean and healthy. The lack of this gene is indeed favourable without any "side effect" raising the question of why p66Shc might have been selected, and what is its physiological role. Caloric restriction and cold affect early survival of the species and fecundity while reproduction is a high energy-cost process that relies on energetic and endocrine fat functions. We hypothesize that p66Shc might play a role in these process. To address these issues we studied survival, reproduction and fecundity of p66Shc-/- mice both in a population living in an outdoor enclosure - exposed to food competition and winter temperatures - and under controlled laboratory conditions - with specific regard to maternal behaviour -. Results show that under natural conditions the deletion of p66Shc is strongly counterselected while in the laboratory p66Shc-/- mice have deficits in fat and reproductive behaviour. These findings indicate that p66Shc has been conserved through evolution because of its role in energy metabolism, and caution should be exercised against premature conclusions regarding gene functions which have only been observed in controlled laboratory conditions.

Profiling trait anxiety: Transcriptome analysis reveals cathepsin B (Ctsb) as a novel candidate gene for emotionality in mice

L. Czibere[1], L.A. Baur[1], A. Wittmann[1], K. Gemmeke[1], A. Steiner[1], P. Weber[1], B. Pütz[1], N. Ahmad[1,2], M. Bunck[1,3], C. Graf[1,2], R .Widner[1], C. Kühne[1], M. Panhuysen[3], B. Hambsch[1], G. Rieder[4], T. Reinheckel[5], C. Peters[5], F. Holsboer[1], R .Landgraf[1], J.M. Deussing[1,2]

[1]*Max Planck Institute of Psychiatry, Munich, Germany;* [2]*Helmholtz Zentrum München, Institute of Developmental Genetics, Neuherberg, Germany;* [3]*Affectis Pharmaceuticals, Martinsried, Germany;* [4]*Max von Pettenkofer Institute, Ludwig Maximilians University, Munich, Germany;* [5]*Institute of Molecular Medicine and Cell Research, Faculty of Biology, Albert Ludwigs University, Freiburg, Germany*

Behavioral endophenotypes are determined by a multitude of counteracting but precisely balanced molecular and physiological mechanisms. In this study, we aim to identify potential novel molecular targets that contribute to the multigenic trait "anxiety". We used microarrays to investigate the gene expression profiles of different brain regions within the limbic system of mice which were selectively bred for either high (HAB) or low (LAB) anxiety-related behavior, and also show signs of comorbid depression-like behavior. We identified and confirmed sex-independent differences in the basal expression of 13 candidate genes, using tissue from the entire brain, including Coro7, Ctsb, Mbnl1, Mt1, Slc25a17, Trib2, Zfp672, Stx3, Abca2, Enpp5, Hmgn3 and Pdhb. Additionally, we confirmed brain region-specific differences in the expression of Syt4. Our identification of about 90 polymorphisms in Ctsb suggests that this gene might play a critical role in shaping our mouse model's behavioral endophenotypes. Indeed, the assessment of anxiety-related and depression-like behaviors of Ctsb knock-out mice revealed an increase in depression-like behavior in females. Altogether, our results suggest that Ctsb has significant effects on emotionality, irrespective of the tested mouse strain, making it a promising target for future pharmacotherapy.

The role of Glyoxalase 1 in anxiety-like behavior

M.G. Distler[1], L.D. Plant[2], A.J. Hawk[1], I. Aneas[3], G. Sokoloff3, S.C. Meredith1, M. Nobrega[3], A.A. Palmer[3],[4]

[1]*Department of Pathology,* [2]*Department of Pediatrics,* [3]*Department of Human Genetics,* [4]*Department of Psychiatry and Behavioral Neuroscience*

Anxiety disorders are the most common psychiatric conditions in the United States, affecting 40 million adults each year. Investigating molecular pathways that contribute to anxiety may identify new therapeutic targets. One potential target is Glyoxalase 1 (Glo1), a gene that has been implicated in anxiety in both humans and mouse models. We previously identified a copy number variant (CNV) among inbred mouse strains that includes the gene Glyoxalase 1 (Glo1). We used gene expression microarrays and quantitative real-time PCR to establish that the Glo1 duplication is positively correlated with increased Glo1 expression. We tested mice in the open field test to establish that the duplication is also positively correlated with increased anxiety-like behavior. In order to establish a causal role for Glo1 in anxiety-like behavior, we have created BAC transgenic mice that overexpress Glo1. Transgenic mice show an increase in anxiety-like behavior. Currently, we are using these mice to investigate the mechanism by which Glo1 increases anxiety. Although Glo1's biochemical mechanism has been extensively studied, its role in neuropsychiatric conditions has been controversial. Glo1 detoxifies methylglyoxal (MG), a byproduct of glucose metabolism. We are currently investigating MG's behavioral and cellular effects to establish its role in regulating anxiety-like behavior.

Funding support: 5R01MH079103 (AAP) and 5T32GM07281 (MGD)

15:30 -17:30 ***Symposium 4: Zebrafish, a novel model organism in neurobehavioural genetics.***

Chair: R Gerlai

Dopamine modulates Akt signaling and alters GABAergic neuron development and motor behaviour in zebrafish larvae

B.R. Souza[1], M.A. Romano-Silva[2], V Tropepe[1]

[1]*Department of Cell & Systems Biology, Centre for the Analysis of Genome Evolution and Function, University of Toronto, Toronto, ON, Canada;* [2]*Laboratório de Neurociência, INCT de Medicina Molecular, Departamento de Saúde Mental, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil*

An imbalance in dopamine neurotransmission is a hallmark physiological feature of many neuropsychiatric disorders. Dopamine D2 receptors modulate the activity of the protein kinase Akt, which is known to be down-regulated in the brain of patients with schizophrenia. Akt has an important role in the regulation of cellular processes that are critical for neurodevelopment, including gene transcription, cell proliferation and neuronal migration. Thus, it is possible that altered Akt-dependent dopamine signalling itself may lead to defects in neural circuit formation during development. Here we show that D2 receptor activation acutely suppresses Akt activity by decreasing the level of pAkt(Thr308) in the larval zebrafish brain. This D2-dependent reduction in Akt activity negatively regulates larval movement and is distinct from a D1-dependent pathway with opposing affects on motor behavior. In addition, we show that D2-dependent suppression of Akt activity causes a late onset change in GSK3-beta activity, a known downstream target of Akt signaling. Finally, altering the levels of D2 receptor signaling, or reducing Akt activity directly, causes a significant decrease in the number of GABAergic neurons throughout most of the brain. Our observations suggest that D2 receptor signaling suppresses Akt/GSK3-beta activity, which regulates GABAergic neuron development and motor behaviour.

Funding support: Department of Foreign Affairs & International Trade (Canada); Ontario Ministry of Research & Innovation (Canada); and NSERC (Canada).

Visual Behavior Testing in Larval and Adult Zebrafish

Stephan C.F. Neuhauss and Kaspar P. Mueller
University of Zurich, Institute of Molecular Life Sciences; Neuroscience Center Zurich and Center for Integrative Human Physiology, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

The zebrafish (Danio rerio) is a powerful model organism to study the development and function of the visual system. For larvae a number of behavior based visual tests are available, while there is still a lack of behavioral tests for adult fish.
A number of behavioral tests have been used for automatic and large scale testing of larval vision. These include the optokinetic response assay (OKR), visual motor response assay (VMR), and the optomotor response (OMR).
We have adapted the optokinetic response assay (OKR) and the visual motor response assay (VMR) for adult fish, allowing for a fast and quantifiable evaluation of visual function. Additionally we have automated the optomotor response assay (OMR) by using video tracking, allowing for a more precise and less time consuming quantification of this behavior. To complement these assays, we sought for an additional method independent of motion vision (as OKR and OMR are), but allowing a more versatile evaluation of visual system function than merely the reaction to increments or decrements of illumination (as it is the case for the VMR). Visually guided choice discrimination training satisfies these conditions. We automated this method by using video tracking, stimulus presentation on LCD screens and food delivery by electromagnetic valves, to drastically reduce the time investment for these learned behaviors.
The utility of these behaviors will be demonstrated by showcasing current vignettes of our research.

A behavioural function for adult neurogenesis in zebrafish?

W.H.J. Norton[1], K.J. Webb[2], J. Ninkovic[3], J. von Trotter[1], M. Chaminade[1], L. Bally-Cuif[1]
[1]*Zebrafish Neurogenetics, NED, INAF, UPR2197, CNRS, Gif-sur-Yvette, France,* [2]*Deussing laboratory, MPI for Psychiatry, Kraepelinstr.* [2]*-10, 80804, Munich, Germany,* [3]*Goetz laboratory, Institute for stem cell research, Helmholtz Centre Munich, Ingolstaedter Landstr.* [1]*, 85764, Munich, Germany.*

Adult neurogenesis, the process of continuously producing new neurons in the brain, has been documented in many vertebrates. However, the function of new-born neurons and the extent to which they are integrated into existing neural circuits remains unclear. Adult zebrafish show neurogenesis in multiple brain areas including the telencephalon, hypothalamus, preoptic area, optic tectum, and cerebellum. These new-born cells most likely contribute to brain growth, but may also play a role in the control of a number of behaviours. Work in our laboratory has established protocols to measure aggression, boldness, exploration and reward in adult zebrafish. Analysis of novel zebrafish mutants that show either reduced reward behaviour (no addiction) or increased aggression, boldness and exploration (spiegeldanio; fgfr1a) suggest that the areas of the brain critical for the control of these behaviours are also important sites of adult neurogenesis. In this presentation, I will focus on our current research which uses adult zebrafish to explore the link between adult neurogenesis and the control of complex behaviours. Specifically, we aim to manipulate neurogenesis in adult zebrafish and assay any ensuing changes to behaviour.

Using zebrafish to identify developmental and genetic mechanisms contributing to drug dependence and psychiatric disease.

L. Annan, C-S. Chang, J.N Gaviria., M.O Parker. and C.H. Brennan.
Biological and Experimental Psychology Group, School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS.

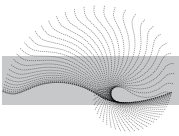
The ability to visualise the establishment and functioning of neuronal circuits in live embryos, and to perform forward genetic and pharmacological screens makes zebrafish an ideal system in which to examine the developmental and genetic mechanisms underlying behavioural disease such as drug dependence and cognitive disorders. In order to exploit the potential of zebrafish in this area we have developed behavioural assays of drug dependence and cognition in adult zebrafish. Using simple associative learning assays we demonstrate that adult zebrafish develop compulsive drug seeking behaviour on repeated exposure to ethanol, nicotine or cocaine, and that drug seeking can be reinstated following withdrawal by stimuli that induce relapse in humans. Further, we show that developmental exposure to either ethanol or nicotine leads to changes in adult behaviours including increased sensitivity to the reinforcing effects of drugs of abuse, altered aggression and shoaling behaviours, and altered performance in positive reinforcement associative learning paradigms. We are now using our assays to screen lines of wildtype and mutant zebrafish, generated through collaboration with the Sanger Institute, UK, to identify developmental and genetic factors contributing to sensitivity to the rewarding effects of ethanol and nicotine and vulnerability to drug dependence and relapse.

Funding support: Medical Research Council UK G1000403. National Committee for the Replacement Refinement and Reduction of Animals in Research G1000053

Genetic and neurobiological mechanisms of alcohol in the brain: zebrafish a novel research tool

Y. Pan, D.Chatterjee, R. Gerlai
University of Toronto, Department of Psychology

The zebrafish has been long utilized in developmental genetics but more recently it has also been employed in the analysis of neurobiological phenomena. Given the powerful genetic tools available for zebrafish, neurobehavioral genetic studies have a good promise with this species. Drug addiction in general and alcohol abuse in particular represent enormous unmet medical needs. The mechanisms of these diseases have started to be analyzed using zebrafish as a model organism. In the current paper we summarize our latest results for the analysis of the effects of acute and chronic alcohol exposure in zebrafish. Using a 3 x 2 experimental design, we investigate the effects of 3 acute alcohol doses and 2 chronic alcohol doses in all combination on behavior, neurochemistry and gene expression in the brain of zebrafish. We also compare genetically distinct populations of zebrafish. Our results show significant acute and chronic alcohol exposure as well as alcohol withdrawal induced changes in behavior, neurochemistry and gene expression. We also demonstrate that many of these alcohol induced changes are strain dependent. Our results show that alcohol has robust effects on zebrafish. The correlated changes between behavior, neurochemistry and gene expression demonstrate that the mechanisms of alcohol induced functional changes in the brain may be investigated successfully. The simplicity of alcohol delivery, the cost effective aspect of zebrafish husbandry, together with the sophisticated genetic, neurobiological and now behavioral tools available for this species, suggest that zebrafish will significantly facilitate neurobehavioral genetic research.



Saturday 14th May 2011

Saturday 14th May 2011 (Aula Pocchiari)

8:30-9:30 Keynote Lecture

How to find quantitative trait genes in the mouse

Jonathan Flint
Wellcome Trust Centre for Human Genetics

Over the past 15 years, more than 2,000 quantitative trait loci (QTLs) have been identified in crosses between inbred strains of mice and rats, but less than 1% have been characterized at a molecular level. However, new resources, such as chromosome substitution strains and the proposed Collaborative Cross, together with new analytical tools, including probabilistic ancestral haplotype reconstruction in outbred mice, Yin-Yang crosses and in silico analysis of sequence variants in many inbred strains, could make QTL cloning tractable. We review the potential of these strategies to identify genes that underlie QTLs in rodents.

10:00 – 12:00 ***Symposium 5: Novel Insights into CaMKII Function in Learning & Memory from Mouse Genetic Studies.***

Chair: K. Peter Giese

βCaMKII plays a non-enzymatic role in hippocampal synaptic plasticity and learning by targeting αCaMKII to synapses

N.Z. Borgesius, G.M. van Woerden, G.H.S. Buitendijk, N.Keijzer, C.C. Hoogenraad, and Y. Elgersma

Department of Neuroscience, Erasmus Medical Centre, Dr Molewaterplein 50, 3015 GE, Rotterdam, the Netherlands

The calcium-calmodulin dependent kinase type II (CaMKII) holoenzyme of the forebrain predominantly consists of heteromeric complexes of the βCaMKII and βCaMKII isoform. Yet, in contrast to βCaMKII, the role of βCaMKII in hippocampal synaptic plasticity and learning has not been investigated. Using a Camk2b–/– mutant, in which βCaMKII is absent, we show that both hippocampal-dependent learning and Schaffer collateral-CA1 long-term potentiation (LTP) are highly dependent upon the presence of βCaMKII. We further show that βCaMKII is required for proper targeting of βCaMKII to the synapse, indicating that βCaMKII regulates the distribution of βCaMKII between the synaptic pool and the adjacent dendritic shaft. In contrast, localization of βCaMKII, hippocampal synaptic plasticity and learning were unaffected in the Camk2bA303R mutant, in which the calcium-calmodulin dependent activation of βCaMKII is prevented, while the F-actin binding and bundling property is preserved. This indicates that the calcium-calmodulin dependent kinase activity of βCaMKII is fully dispensable for hippocampal learning, LTP and targeting of βCaMKII, but implies a critical role for the F-actin binding and bundling properties of βCaMKII in synaptic function. Taken together, our data provide compelling support for a model of CaMKII function in which βCaMKII and βCaMKII act in concert, but with distinct functions, to regulate hippocampal synaptic plasticity and learning.

The role of kinase activity of Ca2+/calmodulin-dependent protein kinase II in hippocampus- and amygdala-dependent memory

Yoko Yamagata[1,2]
1National Institute for Physiological Sciences, 2 SOKENDAI, Okazaki, Japan

We recently generated a kinase-dead CaMKII (K42R) knock-in mouse to study the specific role of kinase activity of CaMKII separately from its other protein functions, such as those as a calmodulin-binding protein and a multimeric structural protein. By using this mouse, we showed that kinase activity of CaMKII is indeed essential for hippocampal synaptic plasticity and behavioral learning. Since previous studies suggested that CaMKII is involved not only in hippocampus-dependent, but also in amygdala-dependent memory formation, we decided to examine these two types of memory in our kinase-dead CaMKII (K42R) knock-in mouse by using Fear Conditioning. After one-pairing conditioning, wild-type mice showed both contextual and cued fear memory. On the other hand, homozygous K42R mice showed no contextual memory, but cued memory was formed to a certain extent. When the number of paring was increased, K42R mice could not discriminate contextual difference, but wild-type mice could, and cued memory was evident in K42R mice, although to a lesser extent than in wild-type mice. Based on the results, I will discuss the contribution of CaMKII kinase activity in hippocampus- and amygdala-dependent memory formation.

Funding Support: #22500301, Grants-in-Aid for Scientific Research <KAKENHI> from Japan Society for the Promotion of Science, JAPAN

Manipulation of Real-time Memory Traces by inducible protein knockout in the mouse brain

Joe Z. Tsien
Brain and Behaviour Discovery Institute, Georgia Health Sciences University, Augusta, GA 30912, USA

By employing an inducible chemical genetic technique, we rapidly manipulated βCaMKII and βCaMKII activity during various memory stages in transgenic or knockout mice. We find that alternations in βCaMKII and βCaMKII activity in freely behaving mice can produce diverse memory impairment including learning, consolidation, and memory retrieval. Our large-scale neural recordings also reveal that rapid manipulation in CaMKII activity also results in alternations in place cell firing patterns as well as episodic cell encoding patterns. Therefore, combination of inducible protein knockout with in vivo neural ensemble recordings can provide crucial insights into the distinct molecular mechanisms and neural temporal dynamics associated with each unique memory process.

Identifying alphaCaMKII autophosphorylation-dependent and independent mechanisms of memory formation

KP Giese
Department of Neuroscience, Institute of Psychiatry, King's College London, 125 Coldharbour Lane, London, SE5 9NU, UK

alphaCaMKII is the most abundant synaptic protein. Its kinase activity and interaction with other synaptic proteins is primarily regulated by autophosphorylations. Autophosphorylation at threonine 286 (T286) is upstream of most autophosphorylations. We have studied knockin mice that cannot autophosphorylate at T286 and found that these mutants have fully blocked NMDA receptor-dependent synaptic potentiation at hippocampal CA1 synapses after a variety of tetanizations, including the use of multiple tetani. These knockin mutants are also impaired in cued and contextual fear conditioning after a single training trial. However, the knockin mutants can form contextual fear memory after a massed training session. This contextual fear memory formation is hippocampus-dependent, indicating that there is an alphaCaMKII autophosphorylation-independent plasticity in the hippocampus that is sufficient for memory formation after massed conditioning. We found that this plasticity requires local protein synthesis and involves a particular type of synaptogenesis. Our findings indicate that in the absence of strengthening of existing synapses, which is alphaCaMKII autophosphorylation dependent, growth of new synapses, which is alphaCaMKII autophosphorylation independent, can enable memory formation after massed training.

βCaMKII, "immature dentate gyrus" and memory

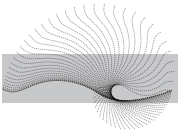
Tsuyoshi Miyakawa[1, 2, 3]

[1]*Institute for Comprehensive Medical Science, Fujita Health University, Japan*

[2]*Center for Genetic Analysis of Behavior, National Institute for Physiological Sciences, Japan*

[3]*JST, CREST, Japan*

Adequate maturation and integration of the adult-generated neurons into the circuit of the hippocampus would be crucial for certain type of learning and memory functions. Disruption of the process could result in some disturbance in mental health. Previously, we reported that mice heterozygous for a null mutation of βCaMKII, a key molecule in synaptic plasticity, have profoundly dysregulated behaviors including hyper-locomotor activity and mood-change-like behavior. Surprisingly, we found that almost all the neurons in the dentate gyrus of the mutant mice failed to mature at molecular, morphological and electrophysiological levels, causing severe deficit in the synaptic plasticity at mossy fiber – CA3 synapses and proposed that "immature dentate gyrus" is a candidate endophenotype of schizophrenia and bipolar disorder (Yamasaki et al., Molecular Brain, 2008; Matsuo et al., Front. Behav. Neurosci., 2009). In this symposium, I will introduce the performance of the mutant mice in learning and memory tasks, such as eight-arm radial maze test, delayed alternation and left/right discrimination tasks using modified T-maze, Barnes circular test and fear conditioning test. The potential link betweenβCaMKII, "immature dentate gyrus" and the learning and memory deficit of the mice will be discussed.



Poster Session

Long term effects of prepuberal subchronic 5HT7 agonist on adult behaviour and excitatory aminoacids in the fronto-striatal interface of Naples High-Excitability rats

L.A. Ruocco[1], F. Zoratto[2], C. Treno[1], U.A. Gironi-Carnevale[1], G. Boatto[3], M. Nieddu[3], A. Sadile[1], G. Laviola[2], W. Adriani[2]

[1]*Department of Experimental Medicine, Faculty of Medicine, Second University of Naples, Naples, ITALY;* [2]*Section of Behavioural Neuroscience, Department of Cell Biology & Neurosciences, Istituto Superiore di Sanità, Rome, ITALY;* [3]*Department of Drug Sciences Analytical Pharmaceutical Chemistry, Sassari University, ITALY*

We investigated behavioural/neurochemical effects of LP211, an agonist of serotonin 7 receptor. In pilot experiment, male Wistar-Han rats were tested for delay intolerance during adolescence (pnd 41-52), while receiving a 5-day treatment with LP211 (0.0, 0.125, 0.250 mg/kg, i.p.), and then for anxiety during adulthood. LP211 0.125 mg/kg slightly affected impulsivity as ongoing subchronic effect, whereas 0.250 mg/kg clearly reduced anxiety as long-term effect. In main experiment, male Naples High-Excitability (NHE) rats, a model for mesocortical ADHD variant, and their random-bred (NRB) controls received prepuberal subchronic LP211 (0.0, 0.125, 0.250, 0.500 mg/kg, i.p., daily pnd 28-42). Rats were tested as young adults (pnd 70-75) in Làt- and Olton-mazes, for activity, non selective and selective spatial attention (SSA). Only in NHE rats LP211 0.125 mg/kg reduced horizontal activity, whereas 0.250 mg/kg increased SSA in the Olton-maze. At sacrifice, excitatory amino acids were evaluated in prefrontal cortex (PFC), dorsal (DS) and ventral striatum (VS). HPLC-MS demonstrated that LP211 decreased L-Glutamate in DS after 0.125 mg/kg and increased it in PFC after 0.250 mg/kg in NHE rats. L-Glutamate was persistently decreased after 0.125 mg/kg only in VS of NRB rats. This new compound is worth to be explored for ADHD therapy.

Funding Support: "under 40" Young-Investigator Project, coordinated as PI by WA, local unit to LAR, Italian Ministry of Health

The cholinergic medial habenula-interpeduncular pathway is critical for impulse control

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The habenular complex links forebrain and midbrain structures and is subdivided into the medial and lateral nuclei. Imaging and histopathologic studies in humans suggest habenular complex dysfunction in schizophrenia and depression. Classic lesion studies in rodents suggest crucial roles of the habenular complex in emotion, learning, stress, attention, and impulsivity. The ventral medial habenula (mHb) expresses specific nicotinic acetylcholine receptor isoforms and releases acetylcholine to the interpeduncular nucleus (IPN), the sole output region of the mHb. The specific function of this circuit, however, remains largely obscure. We generated mice in which cholinergic mHb cells were ablated postnatally. The lesions led to a large reduction in the levels of acetylcholine in the IPN. Although home cage locomotor activity did not differ between the mutant and control mice, the mutant mice showed abnormal adaptation when repeatedly exposed to a novel environment. In a 5-choice serial reaction time test, the mutant micehad a high rate of premature responses, indicative of impulsive behavior. In these tasks, the mutant mice, but not the control mice, were resistant to the effect of systemic nicotine administration. These findings indicate that the cholinergic mHb-IPN pathway is a central circuit underlying impulse control and environmental adaptation.

Compromised decision-making and increased gambling proneness following L-tryptophan dietary depletion in rats

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Impulsive decision-making and exaggerated risk-taking are associated with sensation-seeking and pathological-gambling, both influenced by the brain serotonergic system. We assessed if manipulation of brain serotonin levels affected decision-making and risk-proneness in adult male rats, subjected to an L-tryptophan deficient diet (T+) or control diet (T+; 2.8g/kg). They were tested for decision-making with the rodent Iowa Gambling Task (r-IGT) using home-cage operant-panels. Successively, the same rats were tested for risk-proneness with a probabilistic-delivery (PD) task in operant-chambers. After sacrifice, serotonin and its metabolite were evaluated in selected brain areas. As expected, T+ rats tended to choose the option with best long-term payoff in the r-IGT. They also shifted from large-unlikely to small-sure reinforcers in the PD task. In contrast, T+ animals showed a weaker improvement of performance in the r-IGT and maintained a sub-optimal attraction for the large-unlikely reinforcer in the PD task. HPLC demonstrated drastically reduced brain serotonin synthesis in T- rats. Comparing individual performances in both tests, we found significant correlations within T+ but not T- rats. This may indicate a dissociation between decision-making and risk-proneness, due to an altered function of the serotonergic system. Further studies will focus on neuropsychiatric conditions characterized by gambling and/or disturbed cognitive skills.

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Hidden Markov Model Analysis of maternal behavior patterns in inbred and reciprocal hybrid mice

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Individual variation in maternal care in mammals shows a significant heritable component, with the maternal behavior of daughters resembling that of their mothers. In mice, genetically distinct inbred strains show differences in maternal care during the first postnatal week. In this study we applied a mathematical tool, called hidden Markov model (HMM), to analyze the behavior of female mice in the presence of their young. The frequency of several maternal behaviors in mice has been previously described. However, the ordering, clustering, and transitions between these behaviors have not been described. We used HMM to describe maternal behaviour patterns in two mouse strains, C57BL/6 and BALB/c, and their genetically identical reciprocal hybrid female offspring. HMM analysis is a powerful tool to identify patterns of events that cluster in time and to determine transitions between these clusters. For the HMM analysis we defined 7 states. By quantifying the frequency, composition, and transition probabilities of these states we were able to describe the pattern of maternal behavior in mouse and identify aspects of these patterns that are under genetic and nongenetic inheritance. The differences in these pattern were detected only after the application of HMM analysis whereas classical statistical methods were not able to highlight them.

Prenatal stress and pharmacologic modulation of the endocannabinoid system during adolescence regulate emotional responses and brain metabolism in adult mice

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The central endocannabinoid system (ECS) and the hypothalamic-pituitary-adrenal-axis mediate individual responses to emotionally salient stimuli. Their altered developmental adjustment may relate to the emergence of emotional disturbances. Prenatal stress (PNS) has been shown to upregulate stress and fear responses in adult rodents. Here, we investigated whether PNS – maternal exposure to corticosterone in the drinking water (100 mg/l during the last week of gestation) – combined with a pharmacological modulation of the ECS during adolescence (daily fatty acid amide hydrolase URB597 i.p. administration - 0.4 mg/kg - between postnatal days 29-39), influenced adult mouse emotional behaviour and brain metabolism. PNS adolescent subjects showed reduced locomotion and, opposite to animal facility reared (AFR) controls, were insensitive to the acute effects of URB597 administration. Adult PNS mice showed increased behavioural anxiety and reduced locomotion in the elevated plus maze. Magnetic resonance spectroscopy (VARIAN Inova system operating at 4.7 T) revealed that adult brain metabolism was significantly altered by PNS (hippocampal increased glutamate and reduced taurine; hypothalamic reduced inositol and N-Acetyl-Aspartate) and by URB597 (reduced prefrontal cortex inositol and taurine). Present data further corroborate the view that prenatal stress and pharmacological ECS activation during adolescence persistently regulate emotional responses in adult mice.

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Cholinergic hypofunction in a mouse model of Rett syndrome: beneficial neurobehavioural effects of neonatal choline supplementation

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We studied the long-term effects of a postnatal choline supplementation (from birth till weaning) in the truncated MeCP2-308 mouse model of Rett syndrome. Adult male mutant hemizygous (hz) mice were characterized by a reduced locomotion compared to wild type (wt) littermates. Early choline treatment, via mother drinking water, restored wt-like locomotor activity levels in hz mice. Lower striatal choline acetyl-trasferase (ChAT) activity and decreased levels of cortical mRNA NGF were found in hz mice. Choline supplementation increased striatal ChAT activity and also enhanced NGF and BDNF expression in cortical and hippocampal regions. As a whole, postnatal choline supplementation attenuates some of the behavioural and neurobiological abnormalities of the Mecp2-308 phenotype. Present results suggest that cholinergic depletions in the Mecp2 mutant phenotype should be more extensively characterised and subsequently evaluated for targeted therapeutic approaches. Importantly, choline (or other nutritional) supplementation paradigms appear of higher translational value than those employing enrichment of physical and social environment.

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The effects of early social enrichment on adult resilience to depression-like phenotype are associated with BDNF epigenetic modifications

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During the early postnatal period, the brain is highly plastic and environmental factors play a major role in shaping its structure and function. In order to investigate the effects of the early experiences on adult resilience to major depression and, in particular, to elucidate the molecular mechanisms mediating such effects, we exposed mouse pups to an early social enrichment: the Communal Nest (CN). CN consists in a single nest where three mothers keep their pups together and share care-giving behavior until weaning. When compared to mice reared in standard laboratory conditions (SN), adult CN mice showed endophenotypes of improved resilience to depression, such as more elaborate social competences, reduced anhedonia and lower corticosterone levels following social stress. These behavioral and neuroendocrine modifications were accompanied by higher hippocampal BDNF levels. The epigenetic structure of the BDNF gene was found to be modified accordingly, CN mice having a significantly more acetylated BDNF gene – i.e. a more permissive structure for gene expression -- compared to SN mice. Overall, the present findings suggest a role for BDNF epigenetic modifications in mediating the effects of the early social enrichment on adult resilience to depression.

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Pattern of effects of environmental enrichment during gestation in rats differs by offspring strain, age and sex

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Prenatal environmental enrichment (EE) influences on the behavior of male and female Wistar Kyoto (WKY) "depressive- and anxious-like" rats and their progenitor strain, Wistar rats were examined. Maternal behavior was observed. On weaning day, dams underwent a forced swimming test, and corticosterone (CORT) was measured. The offspring, reared in standard environment, were tested as juveniles (postnatal day [PND] 28-35) or young adults (PND 60-75) in behavioral tests for memory, activity, social, anxiety- & depression- like behaviors. EE decreased dam's depressive-like behavior, while in the offspring EE increased most anxiety-like behavior measures & decreased activity. EE influences were dependent on offspring strain, age and sex. Effects were recognizable at youth more than adulthood, and in WKY less than in Wistar rats. In juveniles effects appeared mostly in males, and in mature animals- mostly in females. EE lowered CORT in young WKY rats' feces. EE induced changes in dams' behavior during pregnancy and lactation which could have mediated offspring outcomes. Thus, prenatal EE may exert long-lasting effects on offspring, which partly decline with maturity. The pattern of influence on the offspring can be opposite from in mature animals, and its' nature, direction and intensity depend on sex and genotype.

Effects of cross fostering on ultrasonic communication in C57BL/6 and BALB/c mouse strains

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C57BL/6 and BALB/c mouse strains differ in levels of maternal care, stress reactivity, and anxiety-like behavior in adulthood. Moreover, BALB/c produce more ultrasonic vocalizations (USVs) than C57BL/6. USVs are influenced by genetic background and environmental factors, but the relative contribution of these factors to such differences remains unclear. We performed a cross fostering procedure between (cross-foster, CF) and within (in-foster, IF) C57BL/6 and BALB/c litters and recorded USVs during brief isolation (PND 8). As adults, mice calling behavior was evaluated in males (courtship behavior) and females (social investigation). In addition, anxiety-like behavior of the adult offspring was measured in the open field. Complete spectrographic analysis on all USVs data was conducted. Results indicated that CF BALB/c pups exhibited a strikingly alteration in their calling profile compared to same strain IF and control groups, whereas CF C57BL/6 pups' vocalizations were not influenced by postnatal manipulation. By contrast, USVs uttered during social encounters were greatly reduced in CF C57BL/6, but unchanged in CF BALB/6 mice. Our findings suggest that USVs production is largely influenced by postnatal environment, but only in the more anxious BALB/c. However, this conclusion is challenged by adult vocalization data, pointing at a dissociation between emotionality and sociability.

Neonatal treatments induce the remote effects in rats and mice, affecting behavior, audiogenic seizure propensity and pain sensitivity.

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Mice and rats of several genotypes were subjected to neonatal injections during first week of postnatal development. The drugs studied were ACTH 4-10 fragment, its analogue Semax, piracetame, caffeine, levetiracetame, busperone etc. Experimental data will be reviewed which demonstrated that the remote consequences of these treatments could be detected in adult mice (CBA, C3H, DBA/2J, 101/HY et al.) and rats (Wistar, WAG/Rij, Krushinsky-Molodkina audiogenic epilepsy strain). These effects were genotype-dependent in many cases. Increase in pain sensitivity in adult mice and rats after neonatal placebo injections were genotype dependent too. Audiogenic seizures proneness in mice and rats changed as the result of neonatal pyracetam, caffeine and levetiracetame et al. injections. The muscular pattern of audiogenic seizures also changed after these treatments. The remote effects discovered were either similar in direction to those of adult animals, or opposite to it. Pharmacological treatments of such type could change some aspects of the CNS development during early postnatal ontogeny presumably changing the development and differentiation of brain neurotransmitter systems. The data on the changes of catholaminergic neurons number in zona incerta after ACTH4-10 and semax could underlie the changes described.

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Maternal Separation is associated with an altered response to stress and epigenetic alterations

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Early life stressors are often implicated in the development of psychiatric disorders. Despite this, the factors that mediate the interaction between gene and environment remain largely unknown. Recent studies provide increasing evidence that such effects are mediated by epigenetic processes. Using a maternal separation paradigm, we investigate phenotypic and epigenetic changes following early life stress in two inbred strains of mice, C57BL/6J and DBA/2J. Early life stress induced a number of behavioral changes, many of which were sex and strain dependent. Changes in anxiety behavior, exploration of novel objects, and baseline activity following maternal separation were observed. Additionally, we found an increase in corticosterone after a stressful event in male, C57BL/6J mice that had undergone maternal separation compared to controls. Finally, we examined genome wide DNA methylation levels in the hippocampus across promoter regions, and compare to levels of gene expression in the same tissue. This study contributes to a growing body of recent literature asserting that epigenetic changes are implicated in translating environmental effects into a behavioral phenotype. Importantly, using two inbred mouse strains allows us to acknowledge the genetic contribution towards both behavioral and epigenetic modifications. Studies such as these will increase understanding of the role of environmental, genetic and epigenetic mechanisms in the development of adverse phenotypes.

Identifying individual differences in the risk for developing alcoholism-related behaviors.

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The development of alcohol use disorders involves a transition from casual use, where alcohol consumption is mediated by its rewarding properties, to alcoholism, in which alcohol-seeking is increasingly controlled by environmental stimuli. Understanding biological factors that predict acceleration of this transition is critical for developing prevention and treatment programs. Here, we used outbred mice to investigate how individual differences in the ability of a food-paired cue to elicit behavior predicted alcohol habit and cue-induced reinstatement. Mice were trained in a Pavlovian-to-instrumental transfer (PIT) paradigm in which they learned to associate a cue with the availability of a food reinforcer, and to perform an instrumental response for the same reinforcer. PIT was assessed by measuring responding in extinction during "Cue On" and "Cue Off" intervals. Mice were then trained to respond for an alcohol reinforcer. Habitual responding was examined using a contingency degradation paradigm. Cue-induced reinstatement was measured after extinction. Data indicated that Pavlovian approach predicted the rapid formation of alcohol habits, while PIT predicted resistance to extinction and greater reinstatement of alcohol-seeking. This model will be used to investigate preexisting risk for the development of alcoholism-related behavior and the genetic and molecular differences that distinguish high- and low-risk populations.

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Differences in anxiety-like behavior in rats selectively bred for high alcohol intake: Rats in the alcohol-preferring P line are less anxious than rats in the high-alcohol-drinking HAD-1 and HAD-2 replicate lines

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Rats selectively bred for divergent voluntary ethanol intake differ in several behaviors. While P rats are more anxious than their non-preferring (NP) counterparts, HAD and low-alcohol-drinking (LAD) rats do not differ. However, few studies have examined differences in anxiety-like behavior among the high drinking lines. In Experiment 1, P and HAD-1 rats had 20-minute access to water or 10% ethanol every other day for 6 sessions and were tested in the elevated plus maze (EPM) following the final session. In Experiment 2, P and HAD-2 rats had 6 weeks of continuous access to 10% ethanol and water and were then either maintained on ethanol or given three cycles of weekly ethanol deprivation and access. Prior to the final ethanol reinstatement, rats were tested in the EPM. In both experiments, P rats showed greater total arm entries and spent more time in the open arms than HAD rats, indicating that P rats are more active and less "anxious" than HAD rats. Furthermore, these genetic differences in behavior were not affected by varying patterns of ethanol exposure, and ethanol intake did not differ between lines. Therefore, P and HAD rats might represent different "typologies" theorized to exist in clinical populations of alcoholics.

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Genomic apoptotic response by neural stem cells to ethanol is shaped by fibroblast growth factor and transforming growth factor β1

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Stem cell vitality is critical for the growth of the developing brain. Growth factors can define the survival of neural stem cells (NSCs) and ethanol can affect growth factor-mediated activities. The present study tested two hypotheses: (a) ethanol causes the apoptotic death of NSCs and (b) this effect is influenced by the ambient growth factor. Monolayer cultures of non-immortalized NS-5 NSCs were exposed to fibroblast growth factor (FGF) 2 or transforming growth factor (TGF) β1 in the absence or presence of ethanol for 48 hr. Ethanol killed NSCs as measured by increases in the numbers of ethidium bromide+ and annexin V+ cells and decreases in the number of calcein AM+ (viable) cells. These toxic effects were promoted by TGFβ1. A quantitative polymerase chain reaction array of apoptosis-related mRNAs revealed an ethanol-induced increase (≥2 fold change; $p<0.05$) in transcripts involved in Fas ligand (FasL) and tumor necrosis factor (TNF) signaling. These effects, particularly the FasL pathway, were potentiated by TGFβ1. Immunocytochemical analyses of NS-5 cells showed that transcriptional alterations translated into consistent up-regulation of protein expression. Experiments with the neocortical proliferative zones harvested from fetal mice exposed to ethanol showed that ethanol activated similar molecular systems in vivo. Thus, ethanol induces NSC death through two distinct molecular mechanisms, one is initiated by TGFβ1 (FasL) and another (through TNF) which is TGFβ1-independent.

Binge-like Ethanol Intake and Concomitant Home Cage Locomotor Activity in Lines of Mice Selected for Sensitization to the Locomotor Stimulant Effects of Ethanol

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Sensitization to the locomotor stimulant effects of alcohol (ethanol) is thought to be a heritable risk factor for the development of alcoholism. However, very little is known about the genetic influences involved in this phenomenon. The first 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, phenotyped for the expression of locomotor sensitization, and bred for high (HLS) and low (LLS) expression of this behavior. A secondary goal was to characterize possible line differences in limited access voluntary ethanol consumption and/or locomotor behavior induced by this consumption. Animals from the second generation of selection (S2) were given daily access to ethanol or water using Drinking-in-the-Dark (DID) procedures while home cage locomotor activity was recorded. Heritability (h2) following 2 generations of selection was 0.50 and 0.18 for the HLS and LLS lines respectively. There were no significant differences in ethanol or water intake between the two lines. However, there were significant locomotor differences between lines in those mice with access to ethanol; LLS animals displaying a relative decrease in locomotion (sedation) and HLS animals displaying a relative increase in locomotion (stimulation).

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Molecular targets of alcohol: From genes to protein-protein interactions

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Recent evidences have shown that alcohol exposure alters the expression pattern of several genes encoding proteins required for normal synaptic function, and suggested that clusters of gene expression profiles correlate with and predict protein-protein interactions. With this study, we aim to define alcohol-sensitive synaptic protein-complexes and to determine if these interactions can be altered by excessive ethanol consumption. We used an interaction proteomics approach, (co-immunoprecipitation, immunoblotting, and LC-MS/MS mass-spectrometry), to identify novel protein interactions in cortical membranes prepared from C57BL/6J mice using calcium-activated potassium channel (BKCa), dynamin-1, synaptobrevin-2 (VAMP-2), and other bait proteins, based on previous gene expression studies. Our results highlight novel important interactions among synaptic proteins with diverse cellular functions, including the dynamin-1 associations with BKCa and VAMP-2. Interestingly, BKCa, SNAP-25, and VAMP-2 share many interacting protein partners encoded by genes whose expression is consistently altered following alcohol excessive consumption. We are currently processing cortices from C57BL/6J mice subjected to Withdrawal Induced Drinking, 2 bottle choice (WID-2BC) paradigm. Using co-immunoprecipitations followed by a semiquantitative mass spectrometric analysis (Isotope Tagging for Relative and Absolute Quantification, iTRAQ), we are testing the effect of the development of alcohol dependence on the identified synaptic protein-complexes: Possible changes may underlie ethanol-related phenotypes.

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Anxiety, alcohol drinking and motor activity: what else lies on rat chromosome 4?

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A locus on rat chromosome 4 was initially found to affect central locomotion in the open field (OF), an index of anxiety, in segregating rats derived from Lewis and SHR strains. Subsequently, this region was shown to also influence ethanol consumption. To isolate and dissect this/these QTL(s), we developed, after 10 generations of backcrossing, a homozygous congenic strain with a segment of chromosome 4 (~27 cM, D4Rat172-D4Rat61) from the Lewis strain inserted into an SHR background. Because the original QTL had shown transgressive segregation, the Lewis alleles were expected to decrease even further the low levels of anxiety found in SHR rats. Ninety four rats (18-28/sex/genotype) were tested at 8-12 weeks of age in the OF, light/dark box (LDB), elevated plus maze (EPM) and activity cages (AC). Congenic rats, when compared with SHR controls, displayed increased: locomotion (central and peripheral) in the OF ($p<0.001$), transitions in the LDB ($p<0.01$), close-arm entries in the EPM ($p<0.001$) and activity in the AC, even after 45 min of habituation ($p<0.05$), thus showing an even less anxious and more active profile than pure SHR rats. Because SHRs are also a model of ADHD, the attention levels of the congenic strain will also be investigated.

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Evaluation of F639A mutation in dNR1 gene as a candidate site influencing ethanol sensitivity in Drosophila melanogaster

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We investigate the sedative effects of alcohol by introducing a phenylalanine to alanine point mutation (F639A) in the NMDA receptor (dNR1) of Drosophila melanogaster. This site has been hypothesized as a functional binding site for alcohol on the NMDA channel. This hypothesis is based on in vitro evidence that the F639A site-specific mutation causes resistance to alcohol's effects in the mouse (Ronald et. al. Ethanol inhibition of N-methyl-D-aspartate receptors is reduced by site-directed mutagenesis of a transmembrane domain phenylalanine residue, J Biol Chem. 276: 44729-44735 2001). To date, no such mutation has been introduced into a model system in vivo. We present our data on the behavioral response to alcohol in this mutant background.

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A gene x environment interaction on alcohol consumption in young adults: the 5-HTTLPR polymorphism and peer's alcohol use.

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Genetic and environmental factors contribute similarly to explain individual differences in non-pathological alcohol use. Among the genetic factors, the serotonin transporter gene (5-HTTLPR polymorphism) has been related to alcohol consumption. On the other hand, peer's alcohol use is one of the strongest environmental factors related to the individual's own drinking. Our main aim was to test the role of the 5-HTTLPR polymorphism and peer's alcohol use on the consumption of young adults. Three hundred and thirty healthy Spanish college students (191 women) were recruited in this cross-sectional study. We genotyped the 5-HTTLPR polymorphism in the whole sample, and assessed the quantity of participants' own consumption and the peer's alcohol use by means of a self-report survey. The ANOVA analysis showed that alcohol consumption in young adults is associated with the 5-HTTLPR polymorphism (SS genotype) and peer's alcohol use. In addition, we found a significant interaction: those participants with the SS genotype that had friends who drink heavily consumed the highest quantities of alcohol. These results indicate that the 5-HTTLPR polymorphism moderates the relationship between peer's alcohol use and individual's own drinking, suggesting that this polymorphism may be considered as a sensitivity factor to risky environments for psychopathology-related behaviours.

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Ethanol drinking microstructure of a high drinking in the dark selected mouse line

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The High Drinking in the Dark (HDID) line differs significantly from the control (HS/Npt) line for the selected measure of blood ethanol concentration (BEC) following drinking in the dark (DID) and also for the g/kg dose of ethanol consumed. However, almost nothing is known about the patterning of drinking in these mice. We examined drinking microstructure in HDID-1 and HS/Npt mice during the 2-day DID test using the BioDAQ Episodic Intake Monitor system, which continuously records the ethanol bottle weight. A bout was defined as a cumulative change in weight greater than or equal to 0.02g, followed by no change for more than a minute. BEC (mg/ml) was determined after a 4 hr drinking session on Day 2. HDID-1 mice drank in more bouts with a shorter interbout interval than HS/Npt, and also consumed more ethanol per bout than HS/Npt while not differing in bout duration. As seen with standard drinking tube studies, HDID-1 mice drank more total ethanol and achieved higher BECs than HS/Npt mice. These results suggest that the difference in BECs between the HDID-1 and HS/Npt animals is driven by fundamental differences in the patterning of drinking, with HDID-1 drinking more frequently and more efficiently than controls.

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Genetic correlates of ethanol drinking in the dark in High Drinking in the Dark murine selected lines

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One limitation of existing rodent genetic animal models targeting alcohol has been that even high-preference genotypes do not consume sufficient ethanol (EtOH) to become intoxicated without procedural manipulation. Therefore, we developed two replicate mouse lines selected for High Drinking in the Dark (HDID-1 and HDID-2) from a heterogeneous control stock (HS/Npt). After 20 (HDID-1) and 13 (HDID-2) selected generations, the lines reach blood ethanol concentrations (BECs) averaging >140 mg% and >80 mg%, respectively, following 4 hr of EtOH drinking early during the circadian dark phase. Studies have now characterized these lines vs HS controls for several responses to acute EtOH administration, as well as EtOH tolerance and withdrawal severity. HDID mice show enhanced sensitivity to some effects, reduced sensitivity to others, and do not differ for yet others. This pattern of idiosyncratic genetic correlation with high DID is consistent with the heterogeneity of EtOH sensitivity established in inbred mouse strains and other selected lines. HDID lines show slightly higher two-bottle preference drinking than HS, and, in a preliminary study, their drinking in limited access sessions escalates more substantially after chronic intermittent exposure to EtOH vapor. The HDID lines provide a new model for initial binge-like drinking.

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Can microRNA explain the polygenic nature of alcoholism?

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Alcoholism has a strong genetic component, however the molecular underpinnings of this debilitating disease are unclear. Recent discoveries point toward a fundamental role of microRNAs (miRNAs) as novel regulators of gene expression influencing almost every biological process. Each miRNA can regulate hundreds of genes. Previously, we established that alcohol alters expression of miRNA 9 (miR-9) in the rat brain causing alcohol tolerance. Recently alcohol regulation of several other miRNAs has been shown. Here we performed microRNA profiling of all known human microRNAs. We used a non-invasive method (saliva collection) to obtain RNA samples from alcohol abusers and non-abusing controls. Results indicate that leukocytes are the main source of RNA in saliva, and expression of a subset of specific microRNAs (including miR-9) is specifically regulated by chronic alcohol consumption. KEGG and DIANA analysis indicate that simultaneous regulation of several miRNAs by alcohol can affect biological pathways important in leukocyte migration and development of several cancers. Together, our findings 1) point to a non-invasive method that provides a fingerprint of human alcohol abuse, 2) uncover novel mechanisms of alcohol actions on the immune system and 3) present a potential new explanation for the polygenic nature of alcoholism.

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Opioid-mediated negative reinforcement contributes to oral self-administration of EtOH.

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While the clinical relationship between stress and drinking has been well substantiated, it has yet to be explained. Scheduling or controlling access to positive reinforcers induces stress (Grant, et al., 2008 for review) and in animal studies has the advantage of taking place in the subject's home cage environment where alcohol (EtOH) can also be made available. In a series of studies involving 2-bottle free choice in the subject's home cage (either 24hr or limited access) we find that blocking access to a running wheel increases self-administration of EtOH. This increased drinking appears to be dependent upon endogenous opioids as it is not evident in b-endorphin deficient subjects. Further, the increase in drinking mediated by blocking access to the exercise wheels is sex dependent (present in females but not in males). These studies support the contention that part of the reason animals find EtOH rewarding is that it ameliorates aversive states by causing the synthesis and release of b-endorphin and suggest that the relationship between stress and b-endorphin may differ in males and females.

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Age and Strain Dependent Differences in Sensitivity to the Aversive Properties of Ethanol: An 8 Inbred Strain Analysis

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The initiation of alcohol (ethanol) use often occurs during adolescence. The many neurobehavioral changes that characterize this developmental period may result in altered sensitivity to ethanol, perhaps leading to a greater propensity for adolescents to consume ethanol. Research has long indicated that alcohol use disorders have a strong genetic component. Unfortunately, thus far there is little research assessing the combined influence of developmental and genetic alcohol sensitivities. Ethanol induced conditioned taste aversion (CTA) has been shown to be highly negatively correlated with ethanol intake in adult mice. Therefore, sensitivity to the aversive properties of ethanol was measured using a CTA procedure during both adolescence (P30-45) and adulthood (P75-90) in 8 inbred mouse strains (C57BL/6J, DBA/2J, 129S1/SvImJ, A/J, BALB/cByJ, BTBR T+tf/J, C3H/HeJ, and FVB/NJ). Adolescent and adult mice were water deprived, and subsequently provided with access to 0.9% (v/v) NaCl solution for 1h. Immediately following access mice were administered ethanol (0, 1.5, 2.25, 3g/kg). This procedure was repeated in 72h intervals for a total of 5 trials. Sensitivity to ethanol induced CTA was highly dependent upon strain; however age-dependent differences were identified in several strains. Future research will continue to assess developmental and genetic contributions to ethanol sensitivity and intake behavior.

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Selection of mice for high cognitive scores task (extrapolation scores). Concommitant changes in anxiety level.

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Inspite of extensive research no attempts were done to select mice or rats for high scjres of cognitive tasks (namely for tasks which require the mental representation, e.g. spatial memory tasks). Laboratory mice ability to extrapolate the direction of movement of the food bait when it disappears from view behind the opaque screen and to find the food using this information was evaluated first in mice of genetically heterogenous population and then in mice specially bred for high scores of this task. The success of extrapolation task solution was scored as the percentage of correct choices during first task presentation (for the group) and as the similar percentage for 6 or 12 trials. The animals with higher scores (correct solution at the 1st trial and no less than 85% of correct solutions in sum) were used for further breeding, their progeny was tested and selected for high extrapolation scores to form the Ex strain. Another criterion for selection was the lack of anxiety behavior during extrapolation test – animals displaying short solution latencies and lack of refusals to participate the task were selected. Heterogenous population mice were randomly bred in parallel with the selected strain and served as non-selected control (Co-Ex). Mice of F1-F4 generations demonstrated no significant differences of correct solution scores from 50% chance level. At the same time the proportion of "good" solvers among mice of selected strain was significantly higher than among controls. The proportion of correct task solutions in mice of F5-F7 was significantly above the chance level – both in selected strain and in control population? No difference was found in the proportion of "good" solvers. At the same time the anxiety levels (elevated plus maze, EPM), stress-reactivity (slippery funnel test as Porsolt test analog) and exploration behavior (closed plus-maze) were different in these Ex and Co-Ex mice. Tx mice were more effitient in exploration of the close plus maze while Co-Ex mice demonstrated the elevated propensity for stereotypical and repeated arm visits together with high defecation scores. The slippery funnel test revealed significantly higher proportion of active coping reactions in Ex mice and their longer duration. The EPM anxiety score were significantly higher in Co-Ex mice. Thus the first generations of mice selection for high "cognitive ability" demonstrated yet not clear differences in the task solution scores per se, but showed the differences in anxiety levels. The hypothesis to be proved or rejected is that cognitive abilities are better revealed when animal anxiety level is nor high, but some optimal level of anxiety could be the necessary prerequisite for expression of cognitive abilities.

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RNA editing and quantifying transcriptomes by RNA-Seq from the prefrontal cortex of ADAR2 transgenic mouse with affective disorder

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RNA editing of the serotonin receptor subtype 2C (5HT2CR) has been implicated in the etiology of affective disorder and obesity. RNA editing is a post-transcriptional process that alters the nucleotide sequence in the primary transcript and a type of Adenosine to inosine (A to I) is observed in 5HT2CR. In higher eukaryotes, family of enzyme known as ADARs catalyzes the adenosine (A) to inosine (I) type of RNA editing in mRNA. Since, inosine and guanosine have the same base pair properties, both transcriptional and the translational machinery reads inosine as guanosine (G).

We examined RNA editing changes of the 5HT2CR and mRNA expression from ADAR2 transgenic mouse that suffers an early onset affective disorder and mature obesity. Pyrosequencing and RNA-SEQ analysis were done on total RNA from prefrontal cortex brain region of control and ADAR2-Tg mice (n=4). Each sample had between 6.3x105 and 7.0x106 mapable reads distributed across the genome using ELAND and between 7.5x105 and 7.2x106 for Bowtie with trimming. The results were analyzed using both ANOVA and DESeq. Using the DESeq model we found 90 genes adjusted p-value <= 0.1. The DESeq list, using geneGo, shows significant GO and pathway term enrichment for developmental processes and cell adhesion.

Interaction between genetic background and chronic stress on neuroendocrine-immune function and depression-like behaviors: implications for tumor progression

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A growing body of evidence reports an association among psychological stress and cancer. We investigated the role of brief -7 - vs. prolonged -21 days - stress as a risk factor for tumor progression. To this aim we compared the effects of restraint stress (RS) and chronic disruption of social hierarchy (SS) on neuroendocrine (corticosterone) and immune (cytokines and splenocyte apoptosis) function in 4-month-old C57 male mice. The effects of SS on depression-like behaviors were also tested. SS subjects did not differ from group housed controls either for depression-like behaviors or neuroendocrine activation. By contrast, when compared to SS, the RS group showed more efficient neuroendocrine and immune responses. In particular, on day 7, we observed reduced corticosterone levels followed by an increase on day 21; these responses were associated to an inverted "U" shape activation of the immune function. When the RS paradigm was applied to a transgenic model of tumor (p53-/- mice) we found reduced lymphocyte apoptosis, in addition to a stronger neuroendocrine activation. These data show a differential role of brief vs. prolonged chronic stress on neuroendocrine and immune function suggesting that the extent of the stress length period might affect tumor progression in different directions.

Wild-derived stock of mice, a useful resource for studying genetic basis of anxiety-like behavior

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The aim of our study is to uncover the genetic architecture underlying individual differences of behavior in animals. In that purpose, we have been studying genetic basis of anxiety-like behavior using a variety of mouse strains including wild-derived strains. In the behavioral studies, we found that the anxiety-like behaviors are largely different among strains. The wild-derived MSM/Ms (MSM) exhibits higher anxiety than C57BL/6 (B6) in open-field test. In order to understand the genetic basis of strain difference in anxiety, we have analyzed consomic strains derived from B6 and MSM. We found that many chromosomes are related to anxiety-like behavior. In the further analyses using the subconsomic strains, which carry short chromosomal segment, we found that a small region on Chr 7 related to differences in the anxiety-like behavior. These results also indicated that the genetic studies on behavioral traits need more sophisticated approach for genome-wide mapping with higher resolution. We are currently making new mouse outbred resource in which a wider genetic repertoire of a panel of wild-derived strains is incorporated. This resource will help better understanding on genetic basis of behavior differences.

Genetic selection for fear conditioning affects anxiety-like behaviors in test and retest of rats in the elevated plus maze

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The elevated plus maze (EPM) is a classic anxiety model that have been widely used in animals. However, the response to anxiolytic drugs has been shown to diminish or even disappear when the animals are previously exposed for one single trial to the same test apparatus, a phenomenon known as "one-trial tolerance". Female rats selectively bred for high/low levels of emotionality were submitted to test and retest to the EPM with a 24 h interval between sessions. These rats are a new line of animals selectively bred for high and low levels of contextual freezing, which has been named Carioca High and Low-Freezing (CHF and CLF, respectively). Female CLF showed decreased measures in anxiety-like behaviors in both sessions. Moreover, prior exposure to the test apparatus produced an overall decrease in the approach towards the open arms in both lines. The fact that genetic differences did not diminish or disappear in the second trial, suggests that differences between naive CHF and CLF rats are not similar to those observed between control and benzodiazepine-treated animals. Furthermore, the similarity of the results found in this study with previous studies using well-known strains of anxiety (Lewis and SHR), supports the use of CLF line in studies of anxiety.

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Wheel running exercise accelerates or delays extinction of conditioned place preference for cocaine in male C57BL/6J mice depending on timing of wheel access

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Exercise is a potential intervention for drug addiction because it promotes brain plasticity and may serve as a substitute reward. However, a danger of incorporating exercise into drug rehabilitation programs is that it may strengthen drug learning. We conducted three studies to determine whether the timing of exercise (i.e. before or after drug exposure) influences the strength of conditioned place preference to cocaine in male C57BL/6J mice. Results across the studies suggest that voluntary exercise strengthens preference for the cocaine-paired texture if exercise is made available prior to drug conditioning. Conversely, exercise appears to weaken preference for the cocaine-paired texture if exercise is made available after conditioning. When exercise is made available after conditioning but one additional drug conditioning session occurs subsequent to exercise, preference for the cocaine-paired texture remains robust in both runners and sedentary animals. There was a significant main effect of exercise condition on neurogenesis in all studies. Results reveal the importance of timing of exercise relative to drug conditioning and the effects of exercise and cocaine on hippocampal neurogenesis.

Dissociation between recent and remote memory: a lesson from mutant mice with dimorphic cortical regions

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The hippocampus plays a crucial role in the formation of novel spatial memories. It has been recently suggested that as these memories mature, they become dependent on extrahippocampal structures, such as the anterior cingulated cortex and the entorhinal cortex. In this study we directly test this hypothesis by using a genetic animal model in which neocortical and archicortical regions are displaced. Cortical deletion of the orphan receptor COUP-TFI, results in a massive expansion of the motor area (Armentano et al., 2007; Tomassy et al 2010). Here, we show that archicortical deletion of COUP-TFI results in a significant misplacement and volume reduction of the hippocampus and the entorhinal cortex, and in a mis-wiring of entorhinal cortical inputs to the hippocampus. Behavioral testing demonstrate that, despite the hippocampal defect, mutant mice are not impaired in the visible and hidden version of the water maze if probed 24 hr after training (recent memory); on the contrary, they are strongly and selectively impaired in the spatial version of the task when tested 35 days after training (remote memory). Taken together these findings suggest that the enthorinal-hippocampus input is not necessary to form novel spatial memories, but to maintain them for longer period.

Experimental evolution of adenylyl cyclase independent learning in Drosophila implicates numerous multi-locus genetic solutions

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One of the great challenges to understanding genetic impact on human disease is that some complex disorders, such as schizophrenia, likely emerge from co-inheritance of multiple common gene variants each of which would have little clinical impact on their own. Despite its widespread relevance, mechanisms by which multi-gene interactions modulate phenotype are ill understood because almost all mechanistic studies of gene interaction are limited to pair-wise studies. To investigate this question, we have developed and implemented a novel approach in Drosophila, using the biologically important and clinically relevant cAMP pathway as a model. We used selective breeding to evolve combinations of alleles capable of suppressing the learning defect of mutations in the rutabaga adenylyl cyclase gene. Unlike a classical suppressor screen, our use of experimental evolution allows us to explore the potential impact of higher order gene interactions. And unlike a classical selective breeding experiment, we constrained the genetic variability to a set of 23 known loci, providing access to the underlying causal alleles. Using independent genetic experiments, we exhaustively tested the effects of each of the identified loci as well as of all di-allele combinations. Our results indicate that numerous genotypic solutions are present and that typical solutions involve combinations of between 3 and 6 loci.

Water-maze performance and adult hippocampal neurogenesis in histamine H1-receptor knockout mice

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The histamine H1-receptor is a G-protein coupled receptor that is expressed in wide parts of the mouse brain including the hippocampus, which is involved in spatial learning. Former studies in H1-receptor knockout mice revealed deficits in a variety of learning and memory tasks. The aim of this study was to analyze negatively reinforced spatial learning of histamine H1-receptor knockout mice in the water-maze and to correlate it with measures of adult neurogenesis.
10 H1R-KO and 12 WT mice were subjected to the following sequence of tests a) cued version, b) place learning, c) spatial probe, d) long-term retention and e) reversal learning. For the analysis of hippocampal neurogenesis 9 H1R-KO and 8 WT mice were investigated regarding cell proliferation, differentiation and survival. H1R-KO mice showed normal cued version, but impaired place and reversal learning as well as long-term retention performance. During the probe trial, they failed to search for the platform in the former platform quadrant. Adult hippocampal neurogenesis is currently under investigation. In conclusion, H1R-KO mice showed marked deficits in spatial learning and memory in the water-maze. Measures of adult hippocampal neurogenesis as potential substrate of the observed cognitive deficits will be presented.

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Prolonged epigenetic regulation of bdnf gene transcription in fear memory

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Gene transcription is required for long-term memory formation. However, the mechanisms that maintain and store memory are not well understood. Recently, epigenetic mechanisms, such as DNA methylation and histone modifications, have been suggested as molecular storage mechanisms underlying memory. Changes in DNA methylation after contextual fear conditioning were reported in genes including brain-derived neurotropic factor (BDNF). BDNF is a growth factor that promotes new synaptic connections and is important for memory formation. However, more studies are required to establish whether or not these epigenetic changes contribute to memory storage. We are studying the changes of DNA methylation in the bdnf gene and the expression of exon-specific bdnf mRNAs associated with contextual fear memory. Specially, we investigate whether these changes are long lasting, not temporally, so they could be molecular storage mechanisms and whether there are sex differences since some transcriptions were previously shown to be sex-dependent. So far, we have identified conditioning-induced changes in DNA methylation in the bdnf gene that are maintained for at least 24 h and overall DNA methylation was regulated in a sex-specific manner in the adult mouse hippocampus. Currently, we are studying which isoforms of the bdnf mRNA gene are regulated after contextual fear conditioning.

Studying Mushroom Body lobe specific STM in Drosophila

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Drosophila melanogaster aversive olfactory memory can be genetically dissected into multiple different phases, including short term memory (STM), middle term memory (MTM), amnesia sensitive memory (ASM), amnesia resistant memory (ARM) and long term memory (LTM). Each memory phase requires overlapping but distinct sets of genes. In the neural circuit level, Mushroom Body (MB) is widely thought as the learning center of the fly brain. Convergent evidences also support a view that different memory phases could also be dissected into separate neural circuits within MB. However, an integrated view of these two dissections has not been established.A recent study in our lab has highlighted the significance of this integrated view. Blum et al. (2009) has shown that Rutabaga function is required in gamma lobe to support STM, but in alpha/beta lobe to support LTM. Based on this observation, Blum et al. (2010) proposed a hypothesis that two parallel STM are formed in MB. The Rutabaga-dependent STM forms in gamma lobe and couldn't consolidate into LTM. In contrast, Rutabaga-independent STM forms in alpha/beta lobe and would consolidate into LTM. In this poster, we are developing genetic tools that allow us to isolate each lobe specific STM and further analyze their feature.

Spatial Learning and Corticosterone Stress Response in 5-HTT Knockout Mice

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Serotonin transporter (5-HTT) knockout mice display a number of phenotypic changes especially regarding anxiety related behavior. 5-HTT is highly expressed in cortical areas involved in cognitive functions, implying also an important role in learning and memory. Moreover, cognitive processes are strongly influenced by emotionality and are modulated by stress-related hormones. In the present study we evaluated whether spatial memory is affected by 5-HTT genotype and differences in the aversiveness of testing conditions. Therefore 5-HTT knockout mice, heterozygous 5-HTT mice, and wild-type controls have been subjected to a 5-day series of repeated trials in either a water maze (WM) or a barnes maze (BM). An additional group of mice was used to measure plasma corticosterone concentrations (CORT) related to the different testing procedures.
5-HTT knockout mice performed significantly worse compared with heterozygotes and wild-types in the WM but not in the BM. Both learning tests led to significantly increased CORT. CORT measured after the WM did not differ from CORT measured after the BM in heterozygotes and wild-types but CORT of 5-HTT knockout mice was noticeably higher after the WM. We suggest that this exaggerated stress reaction contributes to the performance differences between the genotypes that were found in WM learning.

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Modelling neurodegenerative diseases: are our hypotheses correct?

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Dementia in neurodegenerative diseases such as Alzheimer's disease (AD) is often accompanied by behavioural and psychological signs and symptoms of dementia (BPSD). These include delusions, hallucinations, activity disturbance, aggression and agitation, anxiety, depression, circadian rhythm disorders and phobias. Physical aggression and agitation, which occurs in up to 65% of studied patients, are the most common. This behavioural disturbance is physically and emotionally stressful not only to patients but also to immediate family and caregivers, often triggering placement in long-term care facilities. A transgenic mouse model, denoted Tg2576, which over-expresses a mutated human amyloid precursor protein gene implicated in familial AD, was used to investigate aggressive behaviour. The aggressive behaviour of transgenic and control males was evaluated in a resident-intruder test in which a resident male interacted with an experimentally naïve passive intruder male of A/J strain. Tg2576 residents demonstrated significantly higher and unchanged aggression towards intruders placed in their home cages as compared to their non-transgenic littermates. However, the increased territorial aggression of transgenic males needs to be analyzed in the context of general cognitive changes in the model before unequivocal causal links between increased aggression and amyloid pathology can be established.

Visuo-spatial learning and memory in six mouse models of Alzheimer Disease

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The Morris Water maze (MWM) is the most commonly used test for visuo-spatial learning and memory in rodents and is frequently used to test cognitive deficits in mouse models of Alzheimer Disease (AD) (D'Hooge & De Deyn, 2001, Brain Res Rev 36, 60-90). We have tested males and females of five strains of AD model mice (APPInd, APPSwInd, AppSwe/PS1, AppSwe/PS1dE9, 5XFAD) and their wildtype (WT) control strains in the MWM between 3 & 24 months of age and are now testing the 3XTg strain. Both longitudinal and cross-sectional studies were completed. This paper summarizes the effects of genotype, age and sex on measures of learning (latency, search strategy) and memory (percent time in correct quadrant, annulus crossings) in the MWM and discusses background gene effects which influence performance independently of transgene effects. The most significant genotype effects were found in the APPInd and 5XFAD mice and there were few significant sex differences, although females appear to use a different search strategy than males. All strains showed reduced performance with age. Background genes for retinal degeneration and albinism affect performance independently of AD transgenes. The results demonstrate the use of the MWM as an analytical tool in dissociating the factors underlying visuo-spatial learning and memory in AD model mice.

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Conditioned taste aversion in the IntelliCage-validation of the procedure

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Conditioned taste aversion in rodents develops when a novel flavor is associated with sickness. We adapted this biologically meaningful form of classical conditioning to the IntelliCage, a test system that houses groups of mice and automatically records fluid consumption and choice behavior of each mouse.

C57BL/6 (N=68) and DBA/2 (N=58) female mice were kept in mixed groups (N=10-12) and adapted to restricted access to tap water (2x30 min/day). The water was available from 2 bottles in corners of the IntelliCage. On day 8, water was replaced for the conditioned mice (CTA) by a solution containing 75 mM LiCl and 0.5% saccharin. This novel taste solution provided the CS, and the LiCl induced at the same time malaise as the US. Control mice (CON) could drink a solution containing 75 mM NaCl and 0.5% saccharin that did not cause a malaise. Previous experiments proved that mice are unable to distinguish between LiCl- and NaCl-solutions of equal molar concentration. During a choice test mice could either drink water from one or the sweet-and-salty solution from another bottle.

Compared to CON mice, CTA mice drank significantly less of the sweet-and-salty solution. This aversion was independent of housing conditions (CON and CTA mice together or separate) and appeared after 1 as well 2 conditioning sessions.

Further, DBA/2 mice developed a stronger aversion than C57BL/6 mice. Thus, the development of a conditioned taste aversion can be automatically recorded in the IntelliCage.

Behavioural deficits in mice associated with Katnal1 dysfunction

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We are conducting forward genetic screens in ENU mutagenized mice to identify novel circadian phenotypes and the genes underlying them. One of the phenodeviants identified in these screens shows a reduced period length in constant darkness in homozygotes. Mapping and gene sequencing has identified the causative gene for this phenotype as Katnal1. Katnal1 is a member of a family of microtubule severing proteins that also includes katanin p60 and spastin. Since mutations in these other microtubule severing proteins are known to cause neuronal defects, we have behaviourally characterised the Katnal1 mutant to try to establish how the mutation may be affecting neuronal development and function. In contrast with other microtubule severing mutants, the Katnal1 mutant line does not show deficits in motor function. However screening for additional behavioural phenotypes in Katnal1 mutants revealed hyperactivity/low anxiety behaviours and working memory deficits. Moreover, histopathological investigation has shown that Katnal1 mutants have a disorganised hippocampal pathology. Continued investigation of these mice will establish how Katnal1 functions within neurons and how its dysfunction can lead to the behavioural and histological phenotypes that we have described.

Selective Breeding for Increased Home Cage Physical Activity in Collaborative Cross and Hsd:ICR Mice.

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Selective breeding experiments for increased wheel running and open field behavior have identified genetic and neurobiological factors associated with increased voluntary physical activity in mice, but no previous study has directly selected for increased distance traveled in the home cage. Therefore, within-family selection was applied to increase home cage activity as measured by continuous video tracking using two different starting populations, G2:F1 Collaborative Cross (CC) and Hsd:ICR mice. Genetic correlations with distance traveled on running wheels and in the open field were evaluated by mid-parent offspring regression. A significant response to selection was observed in CC but not Hsd:ICR. Wheel running was heritable in both populations but not significantly genetically correlated with home cage activity. Open field was not heritable in either population. We conclude that different genes and neural circuits influence physical activity in the home cage as compared to wheel running or open field. Selective breeding for home cage activity in CC mice warrants further exploration.

Automated motor function phenotyping in mice via voluntary wheel running

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At present, the most commonly used method for assessing motor function in rodents is the Rotarod. To complement Rotarod studies, Liebetanz and Merkler pioneered the use of home cage based running wheels followed by introducing a complex wheel lacking bars to measure motor function in mice (Liebetanz and Merkler, Exp Neurol 2006, 202(1), 217-24). As part of a collaborative European project, Phenoscale, our aim was to automate and validate this approach. In baseline studies at two test centres, differences in coping mechanisms to optimize complex wheel running were detectable in inbred mouse strains including B6N, B6J, C3H, and 129Ola. To further validate the test, we studied wheel-running performance in a number of mouse mutant lines expected to show motor function deficits. Mice with mutations in SNAP25 and Synapsin III demonstrated deficits in a number of complex wheel running parameters. Moreover, for progressive neurodegenerative disease models of Huntingdon's Disease and Amyotrophic Lateral Sclerosis, this method could detect motor deficits, even at presymptomatic stages. Observing voluntary wheel running in combination with a more complex wheel and a further refinement through the addition of a defined resistance in the wheel are currently being assessed to define motor deficits in these mouse strains and mutants.

Effects of Sodium Butyrate on Methamphetamine Sensitized Locomotor Activity

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Repeated exposure to methamphetamine (MA) has been demonstrated to sensitize locomotor response to MA. Exposure to drugs, including MA, may lead to increased histone acetylation, an epigenetic effect resulting in altered gene expression. We conducted two experiments designed to investigate the effect of the histone deacetylase inhibitor, sodium butyrate (NaB), administered at the time of expression or during the acquisition of MA-induced sensitization. MA was administered systemically for 10 days to male C57BL/6J x DBA/2J F1 mice in both studies. To test the effect of NaB on the expression of MA-induced locomotor sensitization, NaB was administered once during testing for MA-induced locomotor activity at the end of repeated treatment. The effect of NaB on acquisition of MA-induced sensitization was tested by administering NaB concurrently with MA over the 10-day acquisition period, but not during the test for expression of MA-induced locomotor sensitization. In the expression study, NaB treatment increased the locomotor response to MA in both acutely treated and sensitized animals. However, treatment with NaB during the 10-day acquisition period produced a significant increase in activity scores in sensitized mice only. These data support a potential role for histone modifications in the development of sensitization to MA.

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A mouse inbred strain survey of cocaine locomotor activation and pharmacokinetics

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Inbred strains of mice are genetic reference populations that represent a rich store of phenotypic and genetic diversity. Inbred strain surveys have historically been used to determine the extent of genetic and environmental influences on complex behavioral phenotypes. However, recent technological advances and the resulting genomic information now available in the mouse have expanded the usefulness of phenotypic data collected from inbred strains. Dense SNP haplotype maps can now be used in conjunction with phenotypic data to map genomic loci (termed Quantitative Trait Loci or QTL) involved in complex phenotypes and also to replicate and narrow previously identified QTL. Our laboratory has recently completed an inbred strain survey in 45 strains for initial sensitivity to cocaine and locomotor response to novelty. The data from these studies show extensive phenotypic diversity and most behaviors exhibit moderate to high heritability, making them amenable to genetic analysis. Using an algorithm for associating phenotypes with haplotype status across the genome, we have identified loci that are linked to the behavioral differences observed among inbred strains. The vast number of SNPs currently available also allows for direct SNP comparisons between haplotype groups to identify potential causative polymorphisms in candidate genes that are present under QTL peaks. We are also collecting gene expression data from all 45 strains from brain regions implicated in addiction. These data will be used for expression QTL (eQTL) mapping to identify cis-eQTL that may overlap with mapped behavioral loci. Finally, we have examined cocaine pharmacokinetics (PK) in the same set of inbred strains to determine the extent to which PK differences explain behavioral output. Our data indicate that pharmacokinetics have some influence on behavioral response to cocaine – particularly in strains at the extremes of the phenotypic distribution. However, PK differences alone do not explain strain variability in locomotor response to cocaine.

Wheel running attenuates age-related changes in hippocampal gene expression

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Normal aging alters expression of numerous genes within the brain. These transcription changes likely contribute to age-associated cognitive decline, reduced neural plasticity, and the higher incidence of neuropathologies. As the aging population increases, the need to identify the mediating factors of brain aging is crucial. One promising intervention to counteract the effects of aging is aerobic exercise. Aged subjects that exercise show enhanced cognitive performance, increased hippocampal neurogenesis and synaptic plasticity. Currently, the mechanisms behind the anti-aging effects of exercise are not completely understood. The presented study conducted a microarray on hippocampal samples from adult (3.5 months) and aged (18 months) male BALB/c mice that were individually housed with or without running wheels for 8 weeks. Results showed that aging altered genes related to chromatin remolding, cell growth, immune activity, and synapse organization compared to adult mice. Exercise was found to modulate many of the genes altered by aging, but in the opposite direction. For example, wheel access increased expression of genes related to cell growth and attenuated expression of immune related genes. Collectively, findings show that late-onset exercise may reverse age-related changes in gene expression and identifies possible systems through which exercise may exert its beneficial effects.

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Genetic and Behavioral Dissections of a Drosophila miRNA locus

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MicroRNAs (miRNAs) are ~21–23 nucleotides small noncoding RNA transcripts that regulate gene expression at the post-transcriptional level. miRNAs regulate gene expression by binding to the 3' untranslated region of target mRNA transcripts to facilitate target degradation or translational inhibition. It has been proposed that miRNAs may play important roles in complex neuronal functions and behavioral traits because of miRNA's ability to coordinate the expression of networks of genes underlying brain structure and cognitive function.
In our study, we identified an individual mutated Drosophila miRNA locus that links to a defective olfactory response phenotype. Using standard Drosophila genetics and mutagenesis method, we generated a series of alleles to confirm a causal relationship between the disrupted expression of the microRNA and the olfaction defect. In addition, we utilized the miRNA "sponge" technique to "soak up" the endogeous miRNA and further characterize the function of this miRNA with spatial and temporal resolution.

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A recent mutation in the C57BL/6J mouse genome causes decreased levels of Gabra2 and results in downstream effects on gene expression and behavior

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Gabra2 expression is controlled by a strong cis QTL in multiple brain tissues and across multiple expression platforms. Higher expression is associated with the DBA/2J (D2) allele in the C57BL/6J (B6) x D2 (BXD) population. Intriguingly, Gabra2 levels vary over 4-fold within BXD strains carrying the B6 allele at this locus. The BXD population includes ~80 strains derived from separate crosses between B6 and D2. The first (BXD1-32) and second set (BXD33-BXD42) were created in the late 1970s and early 1990s, respectively. The third set (BXD43-100) was created in the late 1990s and early 2000s. B6 allele expression drops from the first epoch (11.11 ± 0.15) to the second and third (10.50 ± 0.13 and 10.04 ±0.3, hippocampus respectively). This is consistent with a mutation (likely a CNV) occurring in the B6 parental strain after the late 1970s. This mutation alters behavior and gene expression as a measure of impulsivity and expression of Npy and Penk in the hippocampus are both controlled (LRS > 10) by Gabra2 B6 allelic variation. This new segregating mutation in Gabra2, combined with existing transgenic models, will be a valuable tool for identifying the Gabra2 gene network and its role in complex behaviors and disease.

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RNA-Sequencing reveals striatal transcriptome differences between the Inbred Long Sleep (ILS) and Inbred Short Sleep (ISS) mouse strains.

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Since their development, the ILS and ISS mouse strains have been widely studied as models of acute sensitivity to alcohol. In this study, we hypothesize that striatal transcription differences will provide insight into phenotypic strain differences. mRNA from the whole striatum of adult male, alcohol-naïve ILS and ISS mice (n=3/strain) was quantitatively sequenced (Illumina GAII). Reads (28nt) were aligned to the mouse genome using TopHat, and gene expression was quantified and tested for differential expression and alternative splicing with Cufflinks and Cuffdiff. There were 1219 genes differentially expressed between the two strains, when corrected for multiple testing. Utilizing the online bioinformatics tool WebGestalt, these genes were classified according to Gene Ontology (GO) and each GO category tested for over-representation. Of the 1219 differentially expressed transcripts, 422 had annotations and were used for downstream analysis. The remaining un-annotated transcripts were predicted genes, pseudogenes, or expressed sequences. Top GO categories include: synapse (23 genes, p=3.67e-7), synaptic transmission (16 genes, p=1e-4), and regulation of neurotransmitter levels (8 genes, p=0.0033). Notably, Gad1 and Gad2 (both code for GABA-synthesizing enzyme) are more abundantly expressed in the ILS strain. In addition, many splice variants were over-represented in one strain. For example, of 8 known variants in Gad1 that were tested, 5 were differentially expressed, partially due to alternative promoter use. These results suggest innate differences in striatal signaling, which may contribute to phenotypic differences in response to alcohol between these two strains.

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Receptor-gene cross-talk in the autoregulation of the brain serotonin system: effect of chronic DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) treatment

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The neurotransmitter serotonin (5-HT) has been implicated in various physiological functions and kinds of behavior. The multifunctionality of the 5-HT is due to a great variety of 5-HT receptors. Among large family of 5-HT receptors the particular attention is focused on the 5-HT2A receptor due to its association with pathophysiology of behavior. However 5-HT2A receptor contribution to the autoregulation of the brain 5-HT system remains unclear. Here we found that chronic treatment with agonist of 5-HT2A/2C receptor DOI (1.0 mg/kg, i.p., 14 days) produced desensitization of 5-HT2A receptor without significant changes in 5-HT2A gene expression. At the same time, the increase in the expression of the gene encoding key enzyme of 5-HT synthesis, tryptophan hydroxylase-2 (TPH-2), the increase in TPH-2 activity and 5-HT level and decreased expression of serotonin transporter gene, was found in the midbrain of DOI-treated mice. Despite abovementioned alterations chronic DOI treatment failed to change 5-HT-related behavior. The results provide new evidence on implication of receptor-gene cross-talk in the autoregulation of the brain 5-HT system.

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Genome-wide association for methamphetamine-induced locomotor activity in an advanced intercross mouse line: the benefits of recombination

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Sensitivity to the locomotor activating effects of methamphetamine (MA) shares overlapping neurocircuitry with brain areas associated with reward and may contribute to risk for drug abuse disorders. Individual differences in initial sensitivity to MA are controlled in part by genetic factors; however, identifying genes underlying these differences has proven difficult. We used the power of a C57BL/6J x DBA/2J F2 intercross (n =676) and the precision of a C57BL/6J x DBA/2J F8 advanced intercross line (AIL; n = 570) to identify and narrow quantitative trait loci (QTL) associated with sensitivity to the locomotor stimulant response to MA. Mice were injected with saline (days 1 and 2) and MA (day 3; 2mg/kg i.p.) and distance traveled was measured for 30 minutes. Using an integrated genome-wide association study (GWAS) approach to simultaneously analyze F2 and F8 mice, we identified 8 significant QTL affecting MA sensitivity on chromosomes 1, 3, 8, 9, 11, 12, 15 and 16. After excluding non-polymorphic regions between C57BL/6J and DBA/2J mice, the median 1.5-LOD support interval was 14.92 Mb. The average percent decrease in QTL width between the F2 and the combined analysis was 26%, thus reinforcing the advantages of utilizing highly recombinant populations for QTL mapping studies.

Hypothalamic gene expression profile indicates a reduction in G-protein signalling in Wfs1 mutant mice

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Wolfram syndrome 1 homolog (WFS1) gene is coding protein with unknown function but its deficiency causes neuropsychiatric and neuroendocrine syndromes, including paranoid delusions and severe depression. The hypothalamus is the central region of neuroendocrine control and alterations in this region could cause many of the symptoms seen in WFS1 deficiency. The aim of the present study was to find the functional networks in the hypothalamus which may by related to WFS1 deficiency. We performed gene expression profiling (Mouse Gene 1.0 ST Arrays) in Wfs1 deficient mice (ko) compared to wildtype (wt). In order to define the biological consequences of alterations in RNA levels we performed a gene set enrichment analysis. Modified t-statistics were used to compare groups (wt vs ko). 305 genes were differentially expressed with nominal p-value less than 0.01. FDR adjusted p-values were significant (0.007) only for two genes – C4b (t=9.66) and Wfs1 (t=-9.03). However, several genes related to the G-protein signalling were very close to the FDR adjusted significance. For instance, Rgs4 (regulator of G-protein signalling 4) was down-regulated (-0.34, t=-5.4) in Wfs1 deficient mice. Changes in RGS4 and C4B expression were confirmed by QRT-PCR. In humans, RGS4 is in the locus for bipolar disease (BPD) and its expression is down-regulated in BPD.

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Human QKI is essential for astrocyte survival and the expression of astrocyte-specific genes.

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Linkage, association and expression studies suggest that the quaking homolog, KH domain RNA binding (mouse) gene (QKI), is a candidate gene for schizophrenia (SZC). Previously, functional studies of QKI focused mainly on myelin producing oligodendrocytes, since white matter alterations were implicated in the pathology of SZC. However, QKI is also expressed in astrocytes, the most abundant cell type in the brain, but its function in these cells remains unknown. A growing number of studies suggest that astrocyte dysfunction may play a role in pathogenesis of several disorders of the central nervous system including schizophrenia. Recent studies performed in our group indicated a novel role of QKI as a regulator of interferon induction in human astrocytoma cells (Jiang et al., 2010; PLoS One 5(9): e13079). To investigate whether QKI is important for normal astrocyte function we now silenced QKI and its four splice variants in human primary astrocytes. We observed changes in the expression of few structural and functional astrocytic molecules as well decreased viability in QKI-silenced cells compared to untreated controls. Our data indicate that QKI is essential for normal astrocyte function and therefore, alterations of QKI expression may be one explanation to the astrocyte pathology observed in schizophrenic patients.

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Modulation of the postnatal neurogenesis in mice and rats of different genotypes.

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The goal of the work was to investigate the remote effects of early drug injections which presumably intensify the postnatal cell proliferation in the dentate area in mice and rats. The NOS inhibitor L-NAME and semax- synthetic analogue of ACTH4-10 fragment - were injected to young mice (C3H and 101/HY strains) and rat (Wistar and audiogenic susceptible KM strain). L-NAME (50 мкг/кг), or semax (50 мг/кг) were injected subcutaneously in the nape area daily for 5-7 days during first or second weeks of life. Cell proliferation (the number of new cellular elements) was evaluated by means of immunohistochemical staining using the specific markers for the dividing cells or specific proteins. The cell count was performed using the series of frontal brain sections at the dorsal hippocampal level. The increased proliferation activity in subgranular zone of hippocampal dentate area was demonstrated including the new neurons formation after 7-10 days of injections being finished,. No significant interstrain differences in the numbers of new cells were found.
The remote effects of neonatal treatments mentioned above induced the changes in the behavior of adult animals. These included the increase of open field locomotion and the decrease of exploration behavior indices. At the same time the behavioral indices of anxiety increased in adult animals of both species, at the same time demonstrating the genotype-dependent pattern.
Semax neonatal injection were accompanied by increase of the successful extrapolation task solutions in 101/HY adult mice while L-NAME neonatal treatments increased this ability in C3H mice. The remote genotype-dependent changes in behavior of adult animals after neonatal injections probably only partly depend on increase of neurogenesis.

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Audiogenic postictal catalepsy and the catalepsy proneness in rats of different genotypes

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The physiological state of a rat after audiogenic seizure is characterized by audiogenic post-ictal catalepsy. The rat genotypes investigated were: KM strain (high audiogenic epilepsy, AE, propensity, Wistar rats with two subgroups which either develop low intensity AE fits or are resistant, two new substrains, bred in laboratory from KM-Wistar resistant hybrid population for high and low AE propensity (substrains '4" and "0" respectively), Long-Evans rats and Long-Evans strain rats bred for AE (Long-Evans-Selection, LES) and WAG/Rij strain with "abscence " epilepsy. The post-ictal catalepsy duration correlated significantly with the severity of AE seizures ($r=0,65$, $p<0.001$), with exception of LES and WAG/Rij rat genotypes, while KM rats demonstrated the highest degrees of both traits. The spontaneous catalepsy was revealed in 32% of KM rats. These differences were compared with "pinch-induced" catalepsy developing in the course of successive nape pinches. It was maximal in KM strain (65% rats) and in AE susceptible strain "4", and it increased during 10 successive trials. Wistar rats, Long-Evans and LES animals, as well as WAG/Rij rats were pinch-catalepsy resistant. These data as well as the fact that pinch-catalepsy expressivity interacted with audiogenic post-ictal catalepsy permited to suggest that physiological mechanisms of both cataleptic states partly overlap, although being different in some respects. Haloperidol-induced catalepsy (0.5 mg/kg) was most prominent in KM strain while it was absent in pigmented Long-Evans and LES rats.

The highly developed AE susceptibility in rats of KM strain was presumably accompanied by changes in DA and glutamate neurotransmission, which could be the ultimate cause of catalepsy predisposition. The respective data on DA and NMDA receptor binding in KM and "0" strain will be presented.

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Genetic mechanism of catalepsy in mice: Map3k1, Il6st, Gzmk, and Hspb3 genes coexpression network

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Catalepsy (freezing reaction) is a natural defensive reaction against predator. An exaggerated form of freezing reaction is a symptom of grave brain dysfunctions. The QTL analysis mapped the major gene of catalepsy in mice on the distal fragment of chromosome 13. Catalepsy was shown to be linked to the Map3k1, Il6st, Gzmk, and Hspb3 genes as potential candidates for high predisposition to catalepsy. The genes mRNA levels were measured in the hypothalamus, hippocampus, frontal cortex, striatum and midbrain of catalepsy resistant AKR/J strain and catalepsy-prone strains CBA/Lac, ASC (Antidepressant Sensitive Cataleptic) and congenic line AKR.CBA-D13M76C. The multivariate analysis revealed the interactions between the expression of Map3k1, Il6st, Gzmk and Hspb3 genes in the brain structures. A factor analysis of all variables produced two independent factors explaining 76.2% of the total variance. The catalepsy-resistant AKR strain was distinguished from the catalepsy-prone strains CBA, ASC and AKR.CBA-D13M76C by factor 1. So, factor 1 seems to be associated with genetic predisposition to catalepsy, while factor 2 was not associated with catalepsy, since its values did not significantly differ in parental strains. It was suggested that high predisposition to catalepsy in mice can be defined by Map3k1, Il6st, Gzmk, and Hspb3genes coexpression network.

Association of glycoprotein gp130 with hereditary catalepsy in mice

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Catalepsy is a prolonged motor inhibition, presenting a syndrome of several psychopathologies. Using QTL analysis, selective breeding and genetic recombination, the linkage between Il6st gene encoding glycoprotein 130 (gp130) and catalepsy was shown. The aim was to compare the Il6st gene expression and gp130 sensitivity to lipopolysaccharide (LPS) in mice of catalepsy-resistant AKR/J strain and catalepsy-prone congenic AKR.CBA-D13Mit76 strain with gp130 gene allele transferred from catalepsy-prone CBA/Lac to the genome of AKR/J strain. No difference in gp130 expression in the brain between AKR and AKR.CBA-D13Mit76 mice was found. However, low LPS dose (50 μg/kg) increased mRNA level of gene encoding gp130-regulated glial fibrillary acidic protein in midbrain ($p<0.01$), decreased locomotor activity in open field ($p<0.05$) and reduced level of social behavior and aggression ($p<0.05$) in social investigation test in AKR.CBA-D13Mit76, but not in AKR mice. Moreover, only high LPS dose (200 μg/kg) induced catalepsy in AKR animals, while both doses of toxin (50, 200 μg/kg) increased duration of cataleptic immobility in AKR.CBA-D13Mit76 mice ($p<0.001$). The result indicates (1) association between gp130 and hereditary catalepsy, (2) increased functional activity rather than expression of gp130 in AKR.CBAD13Mit76 mice and (3) involvement of gp130 in the mechanism of LPS-induced alteration of behavior.

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Using a mouse genetic reference panel to identify the genetic basis of cerebellar development and morphology

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We have been using BXD RI, as well as the parental C57BL/6J (B6) and DBA/2J (D2) strains, to understand the genetic basis of cerebellar development and morphology. To understand cerebellar development from a molecular perspective, we have assembled a microarray-based developmental transcriptome for B6 and D2 cerebella across embryonic and postnatal development. RNA was obtained from each strain from each day during embryogenesis (E12-birth) and postnatally up until P9, with gene expression examined using the Illumina microarray platform. We have analyzed these data using novel bioinformatic tools and have identified several novel candidate genes that regulate key developmental events. For example, the G protein-coupled receptor, GPR177, is a candidate gene critical to granule cell development while members of the Notch signaling pathway were shown to be important in cell specification. These data are publicly available on our website (<http://www.cbgrits.org>). To understand the anatomical underpinnings and consequences of these genetic differences, morphological measures were also examined across the same developmental times in the same strains and in adult BXDs with consistent strain differences in cerebellar morphology observed. A significant QTL controlling Purkinje cell number was identified on midchromosome 14, a region containing several interesting candidate genes including cerebellin precursor protein 3.

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Is Methamphetamine Consumption Genetically Related to Consumption of Other Drugs of Abuse?

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To determine whether common genetic mechanisms influence the consumption of multiple abused drugs, we utilized mice that were selectively bred for high and low methamphetamine (MA) consumption (MAHDR and MALDR mice, respectively). In separate studies, two-bottle choice methods were used to measure cocaine and ethanol consumption vs water. In the cocaine consumption study, cocaine solutions were offered at 0.2, 0.4 and then 0.6 g/L concentrations for 4 days each, except for the highest concentration, which was offered for 8 days. During the ethanol consumption study, ethanol solutions were 3, 6, 10 and then 20%, offered for 4 days each. The MAHDR and MALDR mice did not differ in cocaine consumption at any of the concentrations; however, the MAHDR mice consumed more ethanol compared to the MALDR mice. These results suggest that genes that differentiate the MAHDR from the MALDR line do not have an impact on cocaine consumption, but that some common genetic mechanisms underlie MA and ethanol consumption. Similar studies will be performed in a second replicate set of MA drinking lines. Investigation into possible gene sequence and expression differences between these two lines may reveal possible candidate genes which influence both MA and ethanol drinking.

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Signaling Through Mtnr1a / Mtnr1b Melatonin Receptors Effects Nicotine Preference Drinking

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Smoking continues to be the leading cause of preventable death and disease in developed countries and smoking prevalence is increasing in developing countries. Despite high motivation, the lifetime quit rate for smokers is extremely low; only 4%-7%. One potential novel treatment is administration of melatonin which has been shown to reduce cravings in abstinent smokers. Previous studies have shown that sensitivity has a strong diurnal component. and that this change in sensitivity is inversely related to melatonin levels and is dependent upon melatonin signaling. To directly address the impact of melatonin on nicotine intake, knock-out mice that have normal melatonin rhythms but are missing melatonin receptors were assessed for 2-bottle choice nicotine preference. Animals lacking melatonin signaling drank significantly more nicotine. In a separate experiment, melatonin deficient mice were tested in the same 2-bottle choice paradigm with or without melatonin supplementation in the drinking solutions. Mice whose drinking solutions were supplemented with melatonin consumed significantly less nicotine than those animals without supplemented drinking solutions without altering fluid consumption. The combination of these data indicate that melatonin does modulate nicotine intake in a melatonin receptor dependent manner and suggests that melatonin agonists may be a novel treatment for smoking cessation.

Spontaneus nicotine self-consumption and improvement of cognitive alterations in heterozygous reeler mice

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Important reduction of reelin, a neural development and plasticity associated protein, is reported in brains of schizophrenic patients. The latter also consistently engage in tobacco smoking; nicotine thought to alleviate negative behavioural symptoms or cognitive alterations. In mouse brain cortex, nicotine has been shown to reduce reelin promoter methylation thus increasing its transcription. We assessed the preference for a nicotine solution (10 mg/l), in male mice heterozygous (hz) for reelin, a putative model for symptoms related to schizophrenia. A significant preference for nicotine was exhibited by hz mice in six-day free-choice drinking schedule during adolescence (PND37-42). When mice were followed as adults for performance in cognitive tasks, hz mice exhibited a reduced performance during the retention trial in the novel object test. This profile was positively affected by nicotine, in the absence of changes in the wt group. In the reversal-T-maze task, hz mice showed a reduced number of correct responses compared to wt littermates, thus exhibiting a perseverative profile. Again, their performance was improved by previous nicotine self-administration. Present data support the hypothesis of a pre-existing vulnerability --based on haploinsufficiency of reelin-- to behavioural and cognitive disorders and increased self consumption of nicotine.

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Opioid Effects in Mice Bred to Drink Differing Amounts of Methamphetamine

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Methamphetamine abuse has increased in prevalence in the past decade and genetic factors have been implicated as influential in individual susceptibility. Selective breeding in mice to aggregate risk alleles in one case and protective alleles in the other, has produced two replicate sets of mouse lines that consume high (MAHDR) or low (MALDR) amounts of methamphetamine in a two-bottle choice procedure. Qualitative trait locus (QTL) analysis in the replicate 1 MAHDR and MALDR lines identified a QTL on mouse chromosome 10, which mapped in proximity to the mu-opioid receptor gene, Oprm1. Oprm1 was differentially expressed, with MALDR mice having higher expression than MAHDR mice. Additional studies in MAHDR-2 and MALDR-2 lines found no difference in sensitivity to the analgesic effects of the mu-opioid receptor agonist, fentanyl, in thermal nociception assays. However, MALDR-2 mice, consumed more morphine than MAHDR-2 mice in a two-bottle choice experiment, comparing oral self-administration of morphine (0.3 and 0.7 mg/ml in 0.2% saccharin) vs. quinine (0.2 and 0.4 mg/ml in 0.2% saccharin). MALDR-2 mice were also more behaviorally stimulated after acute exposure to fentanyl in a 3-day locomotor activity procedure. These results indicate that there are negative genetic correlations between methamphetamine consumption and opioid consumption and sensitivity.

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Reduced social interaction and BDNF signaling in BTBR T+tf/J strain, a mouse model of autism

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Autism is a neurodevelopmental disorder characterized by social and communication impairments and repetitive behaviors. The inbred BTBR T+tf/J (BTBR) strain, a putative mouse model of autism, exhibits lower social interactions, higher repetitive self-grooming levels and unusual pattern of vocalizations as compared to C57BL/6J strain. The aim of the present study was to compare during adolescence (postnatal days 30-35) male BTBR performances in two different tasks involving investigation of either social cues (same strain partner) or non social ones (inanimate objects). In the social interaction test, BTBR male mice show a reduction of social investigation of the partner, due to a selective reduction of head sniffing, associated to a decreased ultrasonic vocalization emission rate. Strain differences were not evident in the object exploration test. Electrophysiological analysis revealed a significant reduction of BDNF-induced potentiation of synaptic transmission in BTBR hippocampal slices. BDNF and TrkB protein levels measured in the hippocampal region were significantly lower in BTBR as compared to C57BL/6J mice. As a whole, these data confirm the autistic-like phenotype of BTBR mice already at adolescence (selective decreased interest towards social cues and reduced vocalization rate). In addition both biochemical and electrophysiological data point to a decreased BDNF signaling in the hippocampus of this mouse strain.

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Selective Social Deficits in Engrailed-2 mutant mice

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Studies in human populations have identified the homeobox transcription factor ENGRAILED 2 (EN2) as a likely autism susceptibility gene (Gharani et al. 2004; Benayed et al. 2005; Wang et al. 2008; Benayed et al. 2009; Sen et al. 2010). In mouse, En2 serves as a patterning gene of hindbrain and cerebellum, and impacts neurogenesis and development of monoamine systems. Here we report preliminary findings from comprehensive behavioral phenotyping of En2 mutant mice. Assessment of developmental milestones between postnatal days 2 and14 did not reveal any genotype differences between En2 null mutants, heterozygotes, and wildtype littermates. In a 10 minute juvenile reciprocal social interaction test, En2 null mutants exhibited fewer bouts of following and anogenital sniffing as compared to heterozygous and wildtype mice. Adult En2 null mutants failed to show sociability in our 3-chambered social approach task. Olfactory habituation and dishabituation to social and nonsocial odors, self-grooming, anxiety-like behaviors, pain sensitivity, neurological reflexes, and general health parameters did not differ across genotypes. Grip strength was lower in En2 null mutants. Findings from the juvenile reciprocal social interaction and adult social approach tasks replicate and extend the social deficits previously reported for En2 null mutant mice (Cheh et al. 2006). Assessment of sensory, motor and cognitive functions is currently underway.

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A role of strain difference in waveforms of male ultrasonic vocalization for social behavior

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Male mice emit ultrasonic vocalizations (USVs) for female mice during social behavior, and the USVs have a role to attract the female mice. Although the pattern of USVs shows strain differences, influence of the differences for mating behavior have not been fully analyzed yet. We characterized the strain differences of waveforms in male USVs using 13 inbred mouse strains as well as actual behaviors during male-female interaction. ANOVA showed significant strain effects in frequency, duration, and percentage of waveforms. Principle component analysis showed that PC1 related to frequency and duration, and PC2-4 related to each waveform. Interestingly, a wild-derived strain KJR showed the highest scores for PC2-4, and female mice paired with KJR did not emit rejection-related click sounds. We assumed that waveforms of KJR males have positive role in male-female interaction. Therefore, we extracted waveforms in PC2-4 from USVs of KJR and made a sound file "HIGH2-4". As a negative control, we made another sound file "LOW2-4" by extracting waveforms in PC2-4 from low score strains. In playback experiments, female mice were attracted to the speaker playing HIGH2-4 but not LOW2-4. These results highlight the importance of difference in waveforms of USVs for male preference in females.

Modelling vulnerability for the onset of long term social deficit and related neuroendocrine pathways: Developmental exposure to the non-persistent insecticide CPF in mice

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Prolonged exposure to environmental contaminants at apparently non-toxic doses during development represents a major risk factor for neurobehavioural and neuroendocrine functions. Chlorpyrifos (CPF), a non-persistent organophosphate largely used as insecticide acts as a developmental neurotoxicant. In rodents perinatal exposure to CPF impairs neuronal differentiation, synaptogenesis and gene expression. From a behavioural viewpoint, developmental CPF exposure affects primarily affective/emotional responses, possibly interfering with neural systems further than the cholinergic one, such as serotoninergic and dopaminergic transmission. We have previously shown that per os developmental exposure to CPF exerts pro-aggressive effect on social interactions in males, and alters anxiety levels and maternal behaviour in females.

In the present study we mimic more closely the human exposure scenario by administering female mice from gestational day 15 to lactation day 14 with either a standard diet, or a CPF supplemented diet. Results show that, in absence of brain AChE inhibition, CPF-exposed males display enhanced investigative response to unfamiliar social stimuli whereas females show a delayed onset of social investigation and lack of reaction to social novelty. Preliminary data on hypothalamic neuroendocrine markers at adulthood show: in CPF exposed females, decreased expression of hypothalamic neurophysin I (oxytocin precursor), increased expression of Estrogen Receptor alfa (ERα) and decreased expression of Estrogen Receptor beta (ERβ); in CPF males, decreased expression of androgen receptor and increased expression of ERβ. These findings indicate that developmental CPF at doses in the range of supposed human exposure targets expression of neuropeptides involved in modulation of social behaviour and anxiety.

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Evolution of Vitellogenin, a Gene with Roles in Honeybee Social Behaviours

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Vitellogenin (Vg) affects division of labour of worker bees in colonies of the honeybee Apis mellifera, as well as being essential to queen fertility. Vg has many additional pleiotropic effects in workers, queens, and drones. Vg has been proposed to play an essential role in the "reproductive ground plan" hypothesis of the evolution of eusociality in insects. We sequenced Vg in 3 bee subspecies and in 3 sister bee species. Population genetic and functional protein analyses of polymorphisms in Vg demonstrate that it is a fast-evolving gene subject to positive selection at sites important to its lipid-binding function, and that evolutionary rates are higher in eusocial species with large colonies. We briefly discuss these results in relation to several models of the evolution of eusocial insects.

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Key players in genetic control of the brain serotonin system in the regulation of fear-induced aggressive behavior

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This report concentrates on the involvement of genes encoding pivotal members in the brain serotonin (5-HT) system– key enzyme in 5-HT synthesis, Tph2, 5-HT transporter (SERT) and 5-HT receptors in genetic predisposition to fear-induced aggressive behavior. Experiments were carried out with Norway rats selectively bred for 59-60 generations for high level and for the lack of aggressive reaction to man. Considerable differences between highly aggressive and nonaggressive rats were shown. In aggressive rats: 1) 5-HT1A receptor mRNA expression was decreased, and functional correlates of 5-HT1A receptors showed 5-HT1A receptor desensitization; 2) SERT gene expression in the frontal cortex as well as Tph2 gene expression in the midbrain were decreased; 3) 5-HT2C gene expression in the frontal cortex and hippocampus and startle response to 5-HT2C receptor agonist MK-212 were decreased. There were no differences in the expression of the gene and in functional correlate of 5-HT2A receptor. The results favor the idea that key players in genetic control of brain 5-HT system contributes to individual differences in fear-induced aggressive behavior, and increased aggressiveness is associated with genetically defined decrease in 5-HT system functional activity.

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Analysis of a growth QTL affecting emotionality and sociality in chickens

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Domestication of animals, regardless of species, is often accompanied by simultaneous changes in several physiological and behavioral traits (e.g. growth rate and fearfulness). In this study we performed a refined mapping of a growth QTL previously reported to influence emotionality and social behavior in an intercross between White Leghorn layers ("WL") and their ancestor, the red junglefowl ("RJF"). The QTL contains a multitude of genes, several of which have been linked to social behavior (for example the vasotocin receptor AVPR1a), and may have been a primary target of selection during domestication. While the QTL analysis verified the trait "bodyweight", different behavioral variables partly mapped to different locations within the QTL, indicating that different linked genes contribute to the domesticated phenotype. Gene expression analysis of the main candidate genes by means of qRT-PCR, comparing the expression between RJF and WL in selected areas of the chicken brain will be performed to provide further evidence for the role of specific genes in influencing different behavioral traits.

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What electrophysiology teaches us about the pathophysiology of dystonia

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Dystonia is a neurological movement disorder characterized by sustained or repetitive involuntary muscle contractions and abnormal postures. To elucidate the pathophysiology of dystonia, electrophysiological recordings were performed in hyperkinetic transgenic mice generated as a model of DYT1 early-onset torsion dystonia. Abnormal muscle activity, such as coactivation of agonist and antagonist muscles and sustained muscle activation, was frequently observed in these mice. Recording neuronal activity in the basal ganglia under the awake state revealed that spontaneous activity in both the external (GPe) and internal (GPi) segments of the globus pallidus reduced, and showed bursts and pauses. Motor cortical stimulation evoked responses composed of early excitation and subsequent long-lasting inhibition, the latter of which was never observed in normal mice. In addition, somatotopic arrangements were disorganized in the GPe and GPi. In a human patient with cervical dystonia (torticollis), motor cortical stimulation evoked long-lasting inhibition in the GPe and GPi, which is similar to that observed in model mice. Reduced GPi activity induced by the cortico-striatal pathway may cause increased thalamic and cortical activity, resulting in the involuntary movements observed in dystonia.

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Morphological characterization of the hippocampus in Eastern rock elephant shrews: implications for multi-modal sensory processing and behavior

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Eastern rock elephant shrews (Elephantulus myurus) are small mammals of the Order Macroscelidae. We describe here in detail the hippocampus of this species, which shows some unique features. Most striking, the hippocampal formation is of an enormous size compared to the neocortex. The dentate gyrus granule cell layer and CA1 are extremely well developed, whereas the dentate molecular layer is thin and CA3 is short. The hilar polymorphic layer is small, and CA4 cell layer reveals numerous but loosely scattered cells. The mossy fibers in the hilus differ between septal and temporal regions similar to guinea pig. In the septal and mid-septotemporal region, mossy fibers form a dense band of zinc-containing boutons only over the polymorphic layer, en passant boutons to CA4 cells are sparse. Temporally, the hilus is filled with zinc-containing boutons as seen in rodents. Similar to the guinea pig, a bulge of mossy fiber terminals at the distal end of the CA3 pyramidal cell layer bordering CA1 can be observed. Stratum oriens and radiatum are well developed and form a very sharp border towards the subiculum. Granule cells, their dendrites and axons stain lightly for Calbindin. However, Calbindin-positive cells are absent in the septal CA1 pyramidal region, few intensely stained Calbindin-positive cells are present at the border to subiculum. Calbindin-positive cells and dendrites in CA1 similar to rodents can only be found in the temporal region. Immunohistochemistry for Parvalbumin is similar to rodents. Parvalbumin-positive cells are frequent in the subgranular layer of the dentate gyrus and in CA3. In CA1, few positive cells are present. Stratum oriens contains few positive cells, mainly in the deepest aspect, even fewer positive cells are present in the distal stratum radiatum. Intensely stained, coarse dendrites can be observed in the hilus and CA3 region, thin but numerous immunopositive dendrites border the CA1 pyramidal cells apically and basally. Regions with intense mossy fibers show reduced Parvalbumin staining; only few coarse dendrites cross these regions. Elephant shrews depend on extensive multi-modal sensory processing, as vision, olfaction and hearing are equally well developed. The distinct morphological characteristics of the hippocampal formation will be discussed for functional implications.

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Characterization of Social Behaviors Reveals a Novel Ultrasonic Vocalization Deficit in Fragile X Syndrome Mouse Models of Mental Retardation

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The goal of this study was to investigate the social vocalizations of mouse models of Fragile X Syndrome (FXS), the most common inherited mental retardation in people. Those with FXS also have high rates of autistic symptoms. Both mental retardation and autism often create significant problems in verbal communication. It has been shown that mice produce ultrasonic vocalizations (USVs) during social interactions, but the effects of FX mutations on USV production has not been previously reported. In these experiments, it was found that Fragile X knockout (ko) mice displayed a pronounced deficit in total duration of USVs compared to wild-type (wt) mice, even though other measures of social interaction did not show a difference between the two. Knockout mice having at least one copy of a human FMR1 transgene were as vocal as non-transgenic wt mice, confirming the significance of Fmr1 on vocalization. It had been previously shown that seizures were elevated in FX mice, and that the seizures could be suppressed by specific glycogen synthase kinase 3 (GSK3) antagonists as well as lithium. Here it was found that lithium present in the diet increased vocalizations dramatically, suggesting that GSK3 may interact with Fmr1 in a common vocalization pathway.

Living in a dangerous world increases anxiety-like behavior – pathology or adaptation

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Anxiety and anxiety disorders are influenced by both, environmental and genetic factors. One genetic factor under scrutiny for anxiety disorders is the genetically encoded variation of the serotonin transporter (5-HTT). In order to investigate the effects of a threatening environment during early phases of life on mice with varying 5-HTT expression (5-HTT knockout mice), a 'dangerous environment' was simulated by confronting pregnant and lactating 5-HTT +/- females with odor cues of unfamiliar males, indicating the risk of infant killing. The offspring (5-HTT +/+, +/-, -/-) were examined for their anxiety-like and exploratory behavior. Growing up in the 'dangerous environment' induced increased anxiety-related behavior and decreased exploratory locomotion in the offspring, the effects being most pronounced in mice lacking 5-HTT expression. We argue that these alterations in behavioral profile represent adaptive maternal effects that help the individuals to cope with adversity. In principle, such effects of adversity on behavioral profile should not automatically be regarded as pathological. Rather and in accordance with modern evolutionary theory they may represent adaptations, although individuals with 5-HTT genotype induced susceptibility to adversity may be at risk of developing pathologies.

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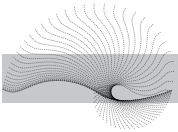

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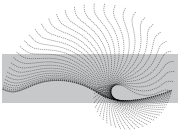

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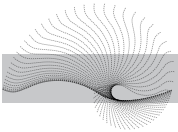

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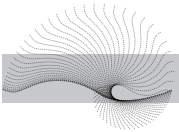

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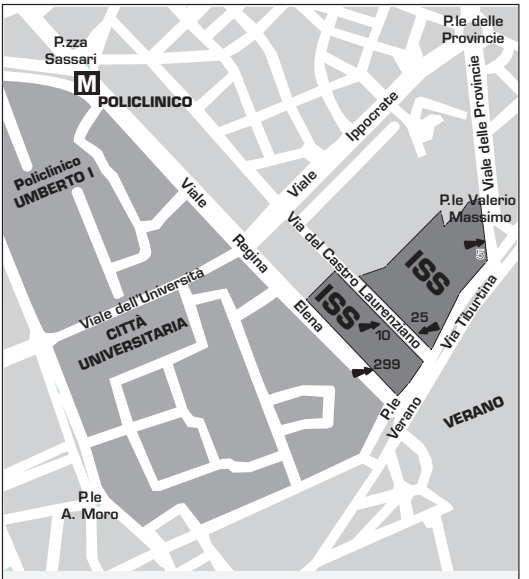
Francesca	**Zoratto**	*francesca.zoratto@iss.it*

Map of the ISS



Entrances:
Via Regina Elena, 299 - Via del Castro Laurenziano, 25
Via del Castro Laurenziano, 10 - P.le Valerio Massimo, 5

BUS: 71 - 163 - 204 - 443 - 448 - 492: "Via Tiburtina-P.le del Verano"; **2 - 3 - 19 - 93:** "P.le del Verano"; **310:** "V.le Ippocrate"
METRO STATION: Linea B: "Policlinico"

Map of Metro


S.Pietro


Pzza di Spagna


Isola Tiberina


Massenzio


Museo Capitolino


Colosseo


Piramide


Castel S.Angelo


Pzza Venezia


Fontana di Trevi


Phanteon


Trastevere


Pzza Navona


Fori

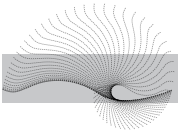

Appia Antica

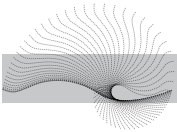

Ostia Antica

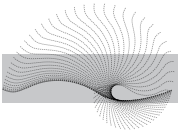

Porta Portese

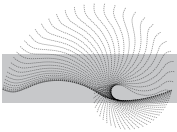

P.za Bocca della Verità

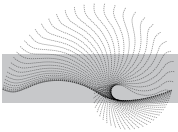

EUR

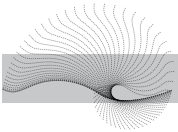


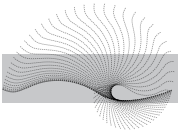


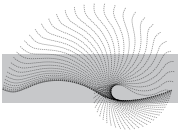


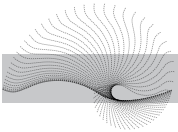


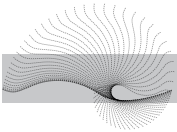


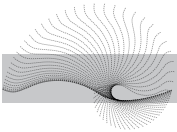












NIAAA National Institute on Alcohol Abuse and Alcoholism
of the NATIONAL INSTITUTES OF HEALTH

NIMH
National Institute of Mental Health