IBANGS Conference Program

Genes, Brain and Behavior

2007 Society Annual Meeting

International Behavioural and Neural Genetics Society
May 21-25, 2007,Golden Tulip Hotel, Doorwerth, The Netherlands

Doorwerth, The Netherlands

Sponsored by:
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Brief Program

Monday, May 21

1.00 – 4.30 pm: Satellite Symposium 1 Sponsored by Neurad

Alzheimer's disease - new insights from animal models and molecular pathways, to be translated into human pathology.

Chairs Thomas Bayer & Fred Van Leuven

A model for a human disease is defined as an experimental preparation developed for the purpose of studying a condition in the same or different species. Typically, models are animal preparations that attempt to mimic a human condition. In developing and assessing an animal model, it is critical to consider and explicit purpose intended for the model. The intended purpose determines the criteria that the model must satisfy to establish its validity. In this context we will discuss different animal models for Alzheimer's disease (AD), which have been developed based on current knowledge of the neurotoxic cascade.

Pathological alterations in the brain of AD patients are characterized by loss of synapses and neurons in specific brain regions leading to its typical clinical symptoms, like memory impairment and change in personality. According to the β-amyloid hypothesis, Aβ peptides liberated from its precursor APP is deposited in Aβ-amyloid plaques, which trigger the pathology, however, plaques don't correlate with the clinical symptoms and loss of neuronal function. Therefore one needs to critically discuss the prevailing β-amyloid hypothesis. The focus of the symposium is to discuss novel ideas of the neurotoxic cascade: evidence for N-truncated Aβ peptides, ratio of Aβ 40/42, intraneuronal aggregation, and role of Presenilin-1 on APP processing. Aβ40 might be even protective by perhaps sequestering the more toxic Aβ42 and facilitating its clearance. Alternatively, there may be a potentially detrimental effect of accumulating APP C99 fragments, which demands further study of the consequences of inhibition of γ-secretase activity. In addition, the complex functional relation of APP and PS1 to cognition and neuronal plasticity in adult and aging brain will be discussed in transgenic mouse models. On the basis of the β-amyloid pathology novel mouse models for AD have been developed. Some of them show a complex pattern of neuron loss, synaptic dysfunction, brain atrophy, behavioural deficits and AD-typical age-dependent pathology. We will discuss the effect of AD-typical pathology on behavioural phenotyping, its effects on exploratory activity, motor coordination, and working memory and other learning paradigms, and the influence of environmental on these measures.

While there may be no perfect animal models for AD, it is clear that each model has strengths and limitations that need to be recognized in order to use the model effectively. The process of developing and validating animal models for AD must work in concert with the process of identifying reliable measures of the human AD pathology and phenomenology.

NEURAD
Neurodegeneration in Alzheimer´s disease
Marie Curie Ph.D. Graduate School

1.00 pm: Samir Kumar-Singh

The mysteries of Aβ amyloid: fibrillar–nonfibrillar, intracellular–extracellular, Aβ40–Aβ42: where does it all lead to?

While losing some of the panache Aβ enjoyed in the Alzheimer's disease (AD) field in the past, it still maintains a dominant position in the disease cascade. This is because no other molecule is as intricately linked to the AD pathogenesis as Aβ. Although this should not deter us from exploring alternative therapeutic possibilities, more efforts should also be put in understanding: (i) the precise steps in the aggregation of Aβ as well as of Aβ aggregation states that are neurotoxic; (ii) other pathological alterations that might be tightly linked to Aβ alterations and in turn contribute to dementia, i.e., altered calcium homeostasis; (iii) the physiological function of Aβ – Aβ42 versus Aβ40 (or Aβ39 etc.) and what does the Aβ40:Aβ42 ratio mean; (iv) the precise compartment intracellular Aβ localizes to and if it is related to dementia as well as cellular alterations noticed in AD patients and in mouse AD models. I will discuss some of these issues in context of some current themes of research being performed in our group. Mostly, I will focus on two things. First, I will discuss some of the recent findings from our laboratory showing that all presenilin mutations studied significantly increased Aβ42:Aβ40 ratio *in vitro*, significantly decreased absolute levels of Aβ40 (with accumulation of APP C-terminal fragments), while only half of the mutations studied significantly increased Aβ42. Interestingly, not only an increase in the absolute levels of Aβ42 or Aβ42:Aβ40 ratio, but also a decrease in Aβ40 levels correlated with the age-of-onset of presenilin 1-linked familial AD. Secondly, I will discuss a novel mouse model expressing APP/Austrian mutation (T714I) – that was earlier shown to cause one of the highest Aβ40:Aβ42 ratio with a remarkable 80% drop in Aβ – to deposit intraneuronal Aβ and reduced brain volumes and fits well with the hypothesis that intraneuronal Aβ could be toxic

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1.20 pm: Benoit Delatour

Behavioral phenotype of a new transgenic mouse model replicating cerebral amyloidosis and neuronal loss of Alzheimer's disease

One major caveat of mice transgenic models of Alzheimer's disease (AD) is their failure to mimic all aspects of human neuropathology. Most of the models reproduce brain amyloidosis (Aβ plaques and amyloid angiopathy). Neuron loss as observed in human disease is, to date, still lacking in most of the currently available mice models. Dramatic loss of hippocampal neurons and severe brain amyloidosis have recently been reported in mice overexpressing mutated APP transgene coupled to knocked-in PS1 mutations linked to familial AD. The aim of our work was to investigate the nature and chronology of behavioural anomalies these APPxPS1-Ki mice are prone to develop. Cohorts including APP/PS1-Ki mice, PS1-Ki cell loss- and amyloid-free mice and wild type mice were, at first, longitudinally phenotyped at 2, 4 and 6 months of age. A first cohort was examined in a battery of neurological tests allowing to measure motor and locomotor functions, sensory abilities and anxiety-related behaviours. Results indicated that, even at very young ages, APP/PS1-Ki showed hyperactivity. These deficits worsened with progressive aging. Cognitive functions were assessed in a second cohort of mice. While short-term retention of information appeared to be preserved in APP/PS1-Ki mice (spontaneous spatial alternation task), gradual deterioration of spatial reference memory, as evidenced in the water maze task, was observed in the same animals. Behavioural analysis of mice trained in other hippocampal-dependent tasks are currently in progress. Finally, to identify which brain lesions (neuron loss, amyloid load, intracellular accumulation of Aβ) are responsible for neurological / cognitive defects, correlative analysis between behavioural performance and morphological markers (neuropathology, biochemistry, brain imaging) will be presented.

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1.40 pm: Kathy Keyvani

Alzheimer's disease: effects of environmental enrichment

Epidemiological studies suggest that the amount of time spent on intellectual and physical activities negatively correlates with the extent of cognitive decline and even risk of developing Alzheimer's disease (AD). Until recently, the most common explanation for this effect was that such lifestyle enhances the cognitive reserve and enables the patients to compensate the clinical expression of AD without affecting AD-related neuropathology. We and others have demonstrated that cognitive and physical stimulation in form of environmental enrichment in transgenic mice does interfere with AD neuropathology and results in significant reduction of cerebral Aβ plaques and in a lower extent of amyloid angiopathy. This effect was independent from APP expression or processing and rather associated with reduced aggregation and enhanced clearance of Aβ. The mechanism appears to be mediated by multiple pathways, in particular a reduced inflammatory response, enhanced microglial phagocytosis, proteasomal degradation, reduced cholesterol levels as well increased angiogenesis and differential regulation of Aβ receptor / transporter molecules promoting Aβ efflux across the blood brain barrier. This talk will give an overview on these findings indicating a hitherto unknown interplay between the environment and Alzheimer diseased brain.

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2.00 pm: Thomas A. Bayer

Synaptic deficits correlate with neuron loss and changes in behaviour in the APP/PS1ki mouse model

The APP/PS1ki mouse model for AD was studied by different means. It expresses human mutant APP751 with the Swedish and London mutations co-expresses two FAD-linked knocked-in mutations (PS1M233T and PS1 L235P) in the murine PS1 gene (named APP/PS1KI mouse model). At the age of six months there is a robust pathology with changes in synaptic function, axonal degeneration, neuron loss due to massive intraneuronal accumulation of N-modified Aβ X-42 peptides. Based on the observation that extracellular Aβ-deposition in typical AD-plaques is not correlated with neuronal degeneration the β-amyloid hypothesis needs a revision with intraneuronal Aβ as the major risk factor for AD.

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2.20 pm: Break

3.00 pm: Chris Janus

Inducible tau mice

Protein tau, a microtubule-associated protein, is implicated in control and integrity of neuronal bidirectional transport. The abnormal accumulation of tau in neurofibrillary tangles occurs during normal aging and is a major pathological hallmark of neurodegenerative diseases known as tauopathies. Consequently, the elimination of toxic tau species through appropriate cellular degradation pathways may prevent or even reverse dementia. A recent study showed that the spatial memory deficits in a conditionally suppressible mouse model of tauopathy could be reversed when the production of tau was significantly lowered (Santa-Cruz, K. et al. 2005, *Science* 309, p.476-81). This mouse model, denoted rTg(tau_{P301L})4510, is characterized by age-related intraneuronal accumulation of insoluble tau and rapidly progressing neuronal death with coinciding cognitive decline.
Since neurodegeneration progresses at different rates in individuals from the same age cohorts, we investigated the relationship between tau levels and memory loss in individual mice. Using cross-sectional design we tested Tg4510 mice in hippocampus-dependent spatial navigation task at ages of 3 months - corresponding to a pre-tangle formation stage, and at 5.5 months, when neurofibrillary tangles appear and a significant loss of neurons in the hippocampus was observed. The results show that the transgenic mice were significantly impaired in spatial memory as compared to non-transgenic littermates, and that this impairment was age progressing. The impairment in spatial navigation manifested itself by inability of the transgenic mice to use spatial strategies during learning acquisition and by poor spatial reference memory. The mice also showed distinctive, age-progressing tau pathology. While hyperphosphorylated tau species were detected in all mice from the 5.5 mo-old cohort, they were observed in only a few mice at the pre-tangle stage. The severity of deficits in the spatial memory of individual 5.5 mo old mice was significantly associated with increased levels of hyperphosphorylated tau species in the brain. The possible mechanism of the cognitive impairment in this mouse model of tauopathy will be discussed in the light of known cellular pathways for the degradation of tau.
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3.20 pm: Paul Lucassen

Increases in LTP and learning but not neurogenesis in young P301L tau mutant mice.

PJ Lucassen, K Boekhoorn, H Krugers, D Terwel, P Borghgraef, M Joels and F van Leuven

In dementia, the hippocampus is particularly affected and extensive tau pathology, aberrant cell-cycle protein expression and changes in neurogenesis have been reported. Neurogenesis has been implicated in hippocampal function and is a.o. stimulated by hippocampal injury. Interestingly, protein tau is implicated in tauopathies like frontotemporal dementia (FTD) but is also important for cell division and neuronal plasticity. Hyperphosphorylation of tau is crucial in the age-related formation of neurofibrillary tangles (NFTs) that correlate well with cognitive decline.
To address the consequences of the P301L mutation itself, we studied tau-P301L mice recapitulating the human phenotype, but at 2 months of age, prior to the onset of tau hyperphosphorylation and axonopathy. Unexpectedly, in these mice, increased long-term potentiation in the dentate gyrus was observed paralleled by an improved memory performance in a hippocampus dependent task. Neither tau phosphorylation, adult neurogenesis, nor other morphological parameters could account for this.
This implies that protein tau is involved in hippocampal function and that not the tau mutation per se, but rather the ensuing hyperphosphorylation must determine the cognitive decline in tauopathies.
Refs; Boekhoorn et al., J Neurosci 2006; Boekhoorn et al., Neurobiol. Dis. 2006.
PJL and KB are supported by the Internationale Stichting Alzheimer Onderzoek (ISAO)
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3.40 pm: Katharina Schindowski

Alzheimer's disease-like cortical and hippocampal imbalance of Brain-Derived-Neurotrophic-Factor and Nerve-Growth-Factor in a tau transgenic model

Katharina Schindowski, Karim Belarbi, Alexis Bretteville, Séverine Bégard, Malika Hamdane, and Luc Buée

We have recently generated the THY-Tau22 mouse model, expressing human
4-repeat tau mutated at sites G272V and P301S under a thy1-promotor that shows Alzheimer¹s disease (AD)-like tau pathology, neurodegeneration, deficits in synaptic transmission and impaired memory. Here we report decreased levels of brain-derived-neurotrophic-factor (BDNF) and increased levels of nerve-growth-factor (NGF) in the hippocampus and cortex very similar to what is reported from AD post mortem brain. BDNF mRNA and protein levels are reduced by 6 months in tau transgenic animals. Interestingly, the loss of BDNF correlates negatively with tau abnormally phosphorylated at Thr212/Ser214 (AT100). In the entorhinal cortex, CA3, DG and to a lesser extent in CA1, we observed neurons that accumulate NGF-immunoreactivity. Interestingly, some of these neurons colocalize accumulated NGF and AT100, but not AT8 (tau phosphorylated at Ser202/Thr205) indicating a crucial role of abnormal phosphorylated tau in this process. Immunoblotting revealed that the levels of pro-NGF were significantly increased in THY-Tau22. However, in contrast to data from AD, we found a significantly increased number of DG neurons expressing NGF mRNA. This is the first mouse model showing an AD-like imbalance of BDNF and NGF in cortex and hippocampus. Since there are growing evidences for the relevance of neurotrophic factor distribution in the pathogenesis of AD, this model is a useful tool to investigate the underlying mechanisms.
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4.00 pm: Fred Van Leuven

Bigenic transgenic mice: amyloid and tau pathology are linked by GSK-3β

The exact recapitulation of pathological processes in brain of AD patients remains a major target for experimental biologists. To this end, we have generated single and bigenic transgenic mice that develop pathological hallmarks of AD. Our APP-V717I transgenic mice progressively present amyloid pathology, characterized by intracellular amyloid, diffuse and senile plaques, vascular deposits, all progressively worsening with ageing. This "late" pathology is preceded by "early" defects in cognition and in hippocampal LTP, which was not rescued by neuron-specific inactivation of Presenilin1 (Moechars et al, 1999; Van Dorpe et al, 2000; Dewachter et al, 2002). Our Tau-P301L mice display morbid and moribund tauopathy with intracellular tau-filaments, resulting in mortality before age 1 year (Terwel et al, 2005) but, surprisingly, preceded by improved cognition at young age (Boekhoorn et al, 2006). Ageing APP-V717I x Tau-P301L bigenic mice (13-18 months) have combined AD-like pathology in hippocampus and cortex ("plaques and tangles"), with more extensive amyloid pathology than the parent APP-V717 mice, and with dramatic enhanced forebrain tauopathy, particularly in hippocampus CA1 (Muyllaert et al, 2006). Remarkably, the Tau-P301L mice die before age 1 year, while the APP-V717I x Tau-P301L bigenic mice survive longer, which is tightly correlated to the alleviation of brainstem tauopathy, which is much less prominent than in the parental Tau-P301L mice. Even more remarkable, also Tau-P301L x GSK-3b bigenic mice have a normalized lifespan relative to Tau-P301L mice, again correlating with strongly reduced brainstem tauopathy. Moreover, the -P301L x GSK-3b bigenic mice are characterized by a dramatic forebrain tauopathy with "tangles in almost every neuron". The combined data corroborate the conclusions that (i) neither amyloid not neurofibrillary tangles are toxic per se, (ii) GSK-3b is a - or the - missing link between amyloid and tau-pathology, (iii) but with important brain-regional differences in its actions.

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4.20 pm: General discussion

4.45 – 6.30 pm: Satellite Symposium 2

Behavioral Phenotyping in the Home Cage: Methods and Tools

4.45 pm: Lucas P.J.J. Noldus

Welcome and introduction

5:00 pm: Berry M. Spruijt

Back to the future: Behavioral testing in the home cage

5:15 pm: Maarten Loos

Genetic encoding of anxiety and impulsivity in inbred strains of mice

5.30 pm: Leonie de Visser

Anxiety measured and validated in the home cage

5.45 pm: Pim Kuurman

Mathematical modeling: from measurement data to phenogram

6:00 pm: Guus Smit, Amsterdam

Phenotyping at large: the Dutch Mouse Phenomics consortium

6:15 Andrew Spink, Wageningen

Demonstration of new tools for behavioral phenotyping, with hands-on experience

6.30 – 8.30 pm: Registration and welcome reception

sponsored by Noldus Information Technology Inc.

Tuesday, May 22

8 am: Breakfast

8.30 am – 10.00 am: Plenary Session

8.45 am: Opening remarks: Tamara Phillips

9.00 am: Presidential plenary lecture: CP Kyriacou

Natural genetic variation in circadian behaviour

CP Kyriacou

The molecular analysis of the circadian clock represents one of the success stories in understanding the gene regulation that underlies complex behaviour. Most of the advances have initially come from forward genetic screens in Drosophila, with the generation of de novo variants by chemical mutagenesis. This has allowed a pragmatic molecular stepwise dissection of the clock components. However, natural clock variants, both inter- and intraspecific, have played a role in not only understanding the selective forces that have shaped the evolution of these sequences, but have also in some instances provided insights into the mechanics of the clock. I will describe some examples of this type of work by focusing on the period and timeless genes in insects.

Department of Genetics, University of Leicester, Leicester, UK

10 am: Coffee

10.30 am – 12.30 pm: Symposium 1

Post genome genetic dissection of behavior,

Chair Josh Dubnau

10.30 am: Kristin Scott

Imaging taste in the Drosophila brain

K Scott

The ability to identify food that is nutrient-rich and avoid toxic substances is essential for an animal's survival. Although olfaction and vision contribute to food detection, the gustatory system acts as a final checkpoint control for food acceptance or rejection. The fruit fly Drosophila melanogaster tastes many of the same stimuli as mammals and provides an excellent model system for comparative studies of taste detection. The relative simplicity of the fly brain and behaviors, along with the molecular, genetic and functional approaches available in Drosophila, allow the examination of gustatory neural circuits from sensory input to motor output. We have utilized a combination of molecular, behavioral and calcium imaging studies to determine the taste ligands that different gustatory neurons detect. These studies demonstrate that taste cells are tuned by taste category and are hardwired to taste behaviors, supporting labeled line encoding of taste information.

Neurobiology, Helen Wills Neuroscience Institute, Univ of California, Berkeley, USA

10.55 am: Bruno Van Swinderin

The Standard Matrix: A novel approach to exploring genetic effects on behavior in Drosophila

B van Swinderen, H Dierick, R Andretic, J Wagner, C Kyriacou, R Greenspan

The traditional approach to identifying gene function in model organisms such as Drosophila relies on a direct correspondence between genetic lesions and phenotypic effects. This approach does not address directly the possibility that many phenotypes may be controlled by genetic interactions. To pursue the hypothesis that genetic architecture is as important a driver of phenotypic change as are individual mutations, we designed a diallel cross of eight "random" P-element mutations (outcrossed to the same wild type background) and tested all 28 trans-heterozygous pairs for a number of behavioral phenotypes, including coordination, sleep, optomotor behavior, phototaxis, geotaxis, and circadian rhythms. None of the 8 mutations on their own were predicted to strongly affect any of these behaviors as heterozygotes, and this was confirmed in general. However, we found several trans-heterozygote pairs of these different mutations that produced significant phenotypic effects for the behaviors. This implied that genetic interactions were causing the phenotypic changes, reflecting a change in genetic architecture as a result of the combined alleles. To investigate the nature of such changes in genetic context, we determined gene expression profiles for all 28 trans-heterozygous pairs. These data allowed us to investigate global changes in gene expression associated with epistasis and additivity as well as differential patterns of expression associated with the significant phenotypic effects. These results suggest considerable flexibility and degeneracy in the genotypic control of phenotypes. We shall focus on the overlap between sleep and circadian rhythms data, as seen by correlated phenotypic and gene expression changes in the matrix experiment. The potential for using our standard matrix as a gene discovery tool will also be discussed.

The Neurosciences Institute, San Diego, California, USA
Funding Support: The Neurosciences Research Foundation.

11.20 am: Josh Dubnau

Suppressor screening by selective breeding

M Cressy, E Kockenmeister, J Dubnau

Genetic dissection of memory has identified several key mechanisms and also has revealed that genes involved are highly conserved across animal phyla. Behavioral screens generally have not reached saturation, however, and genetic modifier screens have been largely infeasible. In the case of developmental biology, such second site suppresser/enhancer screens have played a crucial role in discovering mechanistic interconnections between genes. In the case of memory, most identified genes are "orphans" that have not been placed in mechanistic context. Involvement of the cAMP cascade is a notable exception where interactions among a group of genes are reasonably understood. We are using an alternate strategy to identify enhancers and suppressors of the cAMP pathway. This method relies on artificial selection over the course of multiple generations to screen for informative gene interaction (epistasis) between the cAMP signaling cascade and a new collection of 43 transposon-tagged alleles with known roles in olfactory memory in Drosophila. The fact that all 43 alleles are identified and easily genotyped, has permitted us to follow their co-inheritance over each generation of selection.

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York USA
Funding Support: Beckman Young Investigator.

11.45 am: Robert Williams

Reverse Complex Trait Analysis: or what you can do with sequence data from 16 strains of mice

RW Williams

Forward genetic approaches, including ENU mutagenesis and QTL mapping, are generally initiated by scientists interested in discovering sets of gene variants that modulate interesting and well defined phenotypes. In contrast, reverse genetic approaches start with known gene variants, usually knockouts, and try to discover sets of initially undefined phenotypic effects. The major problem with forward genetic methods (phenotype to genes) is the difficulty of achieving sufficient precision and power to define monogenic effects for traits that are almost inevitably under polygenic control. In comparison, the major problem with reverse genetic methods (gene to phenotypes) is that relevant phenotypes are often unknown and that functional consequences of KOs are highly dependent on genetic background and developmental plasticity. Furthermore, in the case of behavioral traits, the effects of KOs may be easily confounded by allelic variants in neighboring genes--Gerlai's and Crusio's so-called hitchhiker effect (see the work of L. Flaherty). Reverse complex trait analysis is a novel compromise approach that is now possible because we have so much high quality sequence data for multiple strains of mice. The public 8x shotgun sequence for strain C57BL/6J is strongly supported by Celera's shotgun sequence for an additional four strains (A/J, DBA/2, 129S1/SvImJ, 129X1), and by very new NIEHS-Perlegen tiled sequence for yet another 11 strains. For most mouse crosses, such as those made between C57BL/6J (B) and DBA/2J (D) we can readily generate an enormous list of known sequence variants. We can then partition individual mice into genotype classes (e.g., BB, BD, or DD) at a particular functional SNP in a defined gene that is thought to influence a trait. A simple t test or ANOVA can then be used to estimate the p value that may support the association. The advantage of this approach is that we begin with defined sequence variants and with defined phenotypes. A further advantage (caveats apply) is that this method is not burdened as heavily by multiple testing as is standard QTL mapping. The SNP Browser in GeneNetwork (GN, www.genenetwork.org) incorporates almost all known SNPs for 60 or more strains of mice and can filter SNPs by type, location, and potential biological impact. GN also incorporates extensive collections of phenotypes for many common strains of mice and for a small number of common crosses and recombinant inbred reference panels. Some of the reference panels, particularly the extended BXD panel (n of up to 65), are now of sufficient size to provide strong negative results and tantalizing but still provisional positive results.

Center of Genomics and Bioinformatics, University of Tennessee Health Science Center, Memphis, Tennessee, USA
GN is supported by NIAAA, NIDA, NCRR, and NCI.

12.10 pm: General discussion

12.30 pm: Lunch

2.00 – 4.00 pm: Symposium 2

Translational genetic approaches to the study of fear and anxiety

Chairs Abraham Palmer & Andrew Holmes

2.00 pm: Abraham Palmer (Travel Award)

Common and different genetic control of learned fear and innate anxiety-like behavior in mice

AA Palmer

This talk will discuss recent studies that examine the genetics basis of fear learning and its relationship to innate (un-learned) anxiety-like behaviors in both mice and humans. Genetic correlations among selected lines and panels of inbred strains provide insight on these topics in rodents. We have examined learned fear and innate anxiety-like behaviors in populations of mice that were selectively bred for differential fear learning. These studies indicated that these behavioral domains are influenced by common genes. We have also a used panel of consomic mice (B6.A) to characterize fear learning and have then used extant data about anxiety-like behaviors in these strains to locate specific chromosomes that harbor genes that pleiotropically influence both learned fear and anxiety-like behaviors. These data support the existence of a broad genetic construct that has previously been termed "emotionality". The implications for identification of candidate genes that can subsequently be examined in human subjects will also be addressed.

Departments of Human Genetics and Psychiatry, University of Chicago, Chicago, Illinois, USA

2.25 pm: Victoria Risbrough

Translational studies of CRF system contributions to anxiety

VB Risbrough

Affective modulation of the startle reflex has been shown to have translational utility for understanding the neurobiology of anxiety and fear. In animals, Corticotropin Releasing Factor (CRF), a peptide released during stress, has been observed to modulate startle responding, increasing startle magnitude while reducing startle inhibition and reflex threshold. Post-traumatic stress and panic disorder subjects exhibit abnormalities in startle plasticity (exaggerated startle, reduced startle habituation, and reduced PPI) and exhibit dysregulation of the CRF system. Here, two themes will be discussed. The first theme will be our recent studies using pharmacology tools and receptor null mutation mice to dissect the relative roles of CRF1 and CRF2 receptors in startle behaviors. Data will include recent studies testing the hypothesis that forebrain CRF1 receptors are required for stress and CRF effects on startle, results of the effects of CRF1 and CRF2 receptor null mutation on unconditioned anxiety and conditioned fear, and finally, recent data comparing the effects of CRF overexpression across different neural circuits on startle phenotypes. The second theme will be the recent development of a novel anxiety-potentiated startle paradigm, its use in high and low trait anxiety subjects and its relationship to amygdala activation and anxiety-related genes.

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Funding Support: MH074697, MH076850, NARSAD.

2.50 pm: Lukas Pezawas

Epistasis of SERT & BDNF: A Complex Genetic Model of Depression

L Pezawas, AL Goldman, BA Verchinski, VS Mattay, JH Callicott, BS Kolachana, RE Straub, MF Egan, A Meyer-Lindenberg, DR Weinberger

BDNF and SERT, both of which have been associated with psychopathological states, are important genes in brain development and in functions related to memory and emotion. Genetic variations of the BDNF (val66met) and SERT gene (5-HTTLPR) affect the function of these proteins in neurons and predict variation in human memory and in fear behavior. Our previous work has shown that the S allele of 5-HTTLPR affects the integrity, function and connectivity, and presumably development of a neural circuit linking amygdala and rostral anterior cingulate circuitry, a circuitry related to anxious temperament and depression in the presence of environmental adversity. Additionally, we could show that val66met BDNF affects the development and function of brain circuitries (hippocampus, DLPFC) prominently implicated in aspects of cognitive functioning (e.g. working memory). Convergent evidence links BDNF to depression, such as data showing association of the functional val66met BDNF polymorphism with increased risk for mood disorders, for temperamental traits related to mood disorders, and associated increases of BDNF expression after electroconvulsive therapy and antidepressive SSRI treatment. These data implicating a biological interaction of BDNF with 5-HTTLPR-dependent signaling suggest a molecular mechanism that could support an epistatic interaction between the functional variants in these genes in risk for depression. This possibility has been explored to a limited degree in animals genetically engineered to be hypomorphic at both genes. We hypothesized that the "insufficient" met BDNF allele does not translate the S allele effect of 5-HTTLPR and therefore protects the subject from significant changes in subgenual cingulate and amygdala volume, which is reflected in functional connectivity data of this brain circuitry. We investigated high-resolution anatomical magnetic resonance images (MRI) of 111 normal healthy volunteers (Caucasians of European >ancestry) without any psychiatric life-time history using optimized voxel-based morphometry (VBM), a sophisticated fully automated morphological imaging technique, which allows a statistical comparison of gray matter volume on a voxel-by-voxel basis. Furthermore, functional and structural connectivity data were analyzed using SPM2. Consistent with our initial hypothesis, we found a significant increased 5-HTTLPR S allele volume loss of the subgenual cingulate and amygdala ($p<0.001$) in val/val BDNF carriers compared to met BDNF genotype. Structural connectivity and behavioral data reflected this relationship ($p<0.001$). The met BDNF allele may be a protective genetic factor for depression, because it only insufficiently translates 5-HTTLPR S allele dependent structural and functional changes, which are related to depression.

Medical University of Vienna, AUSTRIA
Funding Support: GCAP, NIH,NIMH, IRP.

3.15 pm: Andrew Holmes

Exploring the genetic and neural basis of fear extinction using mouse models

A Holmes[1], K Hefner[1], L Heinz[1], D Goldman[1], C A Hodgkinson[1], N Singewald[2]

Extinction is an active form of learning in which the expression of a conditioned fear response is reduced after repeated experience of the conditioned stimulus in the absence of the unconditioned, aversive stimulus (Pavlov, 1927). Impaired fear extinction is a major symptom of anxiety disorders, such as posttraumatic stress disorder, that have a significant genetic component. Fear conditioning and extinction appear to involve partially distinct molecular and neural circuitry. However, while there is increasing understanding of the neural processes subserving fear extinction, the precise neural circuits, molecules and genes underlying impaired extinction remain incompletely understood. To address this question, we have employed a multi-disciplinary approach to study the basis of differences in fear extinction between inbred mouse strains. Multiple inbred strains were compared for extinction of a Pavlovian fear conditioned response and, for comparison, extinction of conditioned taste aversion and an instrumental response for positive reinforcement. The effects of various drugs with known extinction-facilitating effects (e.g., D-cycloserine, yohimbine, URB597) were compared across strains. In order to identify brain regions differentially activated by extinction learning across strains, c-Fos immunocytochemistry was performed. To explore potential sources of genetically-driven variation driving strain differences in extinction, sequencing was performed on a number of genes implicated in extinction (e.g., brain-derived nerve-growth factor, dopamine D1 receptor, catechol-O-methyl-transferase). The results of these studies could provide novel insights into the pathophysiology of anxiety disorders and serve to improve their therapeutic alleviation.

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Funding Support: NIAAA-IRP and Fonds zur Förderung der Wissenschaftlichen Forschung.

3.40 pm: General discussion

4.00 pm: Break

4.30 – 6.00 pm: Outstanding Young Investigators Awardee Talks

Chair John Crabbe

4.30 pm: Anne Albrecht

Expression of neuropeptide Y and somatostatin in fear conditioned mice

A Albrecht[1], M Schulz[1], C Stoppel[1], H-C Pape[2], O Stork[1]

Inhibitory local circuit neurons control activity and information flow in the amygdala and hippocampus, and are critically involved in the control of fear and anxiety. NeuropeptideY (NPY) and somatostatin (SST) act as co-transmitters in overlapping populations of GABAergic interneurons in these brain areas. While NPY is known for its anxiolytic effects, the role of SST is less well understood and the function and regulation of both peptides in contextual and cued fear conditioning still requires comprehensive evaluation.
Here, expression levels of NPY and SST mRNA in central (CE), lateral (LA) and basolateral (BLA) subnuclei of the amygdala were evaluated with laser microdissection and real time PCR analysis. With SST being generally about 10-20fold more prominent, both peptides showed highest expression in the CE and 2-3 fold lower levels in the LA and BLA. This differential expression was further confirmed by *in situ* hybridisation histochemistry. Six hours after auditory cued and contextual fear conditioning a dissociation of SST and NPY expression levels was observed: while SST mRNA expression was transiently increased in the LA upon cued conditioning, NPY mRNA remained largely unchanged in this region under both conditions. In the BLA and CE, both peptides were regulated only moderately. Our data thus indicate a highly specific regulation of NPY and SST mRNA expression in a subregions of the amygdala likely to contribute to differential information processing in cued and contextual fear memory formation.

[1]Institut für Physiologie, Otto-von-Guericke-Universität Magdeburg, GERMANY
[2]Institut für Physiologie I, Westfälische-Wilhelms-Universität Münster, GERMANY
Supported by Deutsche Forschungsgemeinschaft (SFB426, TPB7) and the state Sachsonia-Anhalt (HWP IF 55).

5.00 pm: Clarissa Parker

Genetics of basal plasma corticosterone levels in the LXS Recombinant Inbred mouse strains

CC Parker, CJ Larson, P Carosone-Link, L Lu, B Bennett

The set of LXS Recombinant Inbred (RI) strains is a large mapping panel consisting of 75 RI strains derived from Inbred Long-Sleep and Inbred Short-Sleep progenitors. This strain set provides sufficient power to detect quantitative trait loci (QTL) contributing to complex traits accounting for as little as 10% of genetic variance. Previous studies utilizing other RI panels and F2 populations have successfully identified QTLs for numerous hormones, but they have primarily focused on identification of QTLs linked to disease states rather than on factors involved in natural variation of hormone levels. In the present study, we analyzed basal plasma corticosterone (CORT) levels in male and female mice between 55 and 105 days of age in a subset of the 75 RI strains. These strains displayed a large range of basal CORT levels (0.0000222 µg/100mL to 3.439284
µg/100mL), transgressing those of the inbred progenitors (ILS mean basal CORT =10.5384 µg/100mL; ISS mean basal CORT =10.9989 µg/100mL), thus supporting the utility of this large panel for mapping traits not selected in the progenitors. Using 4856 SNPs identified in the Wellcome-CTC Mouse Strain SNP Genotype Set, and the mapping algorithm R/qtl; we found two suggestive QTLs correlated with basal CORT levels. In females, a suggestive QTL was found on Chromosome 1 (LOD = 1.80; p = 0.63, accounting for 15**%** of VG), and in males, a highly suggestive QTL was found on Chromosome 15 (LOD = 2.51; p = 0.06, accounting for 19**%** of VG). Future work will focus on finishing analysis on the complete RI panel, as well as identification of QTLs linked to plasma CORT levels following exposure to ethanol and to stress. These findings show that large reference RI panels are a useful resource for mapping complex traits and will be valuable for modeling the genetics of complex diseases in human populations.

Institute for Behavioral Genetics, University of Colorado, USA
Funding Support: F31AA016261, U01 AA 014425.

5.30 pm: Heidi Lesscher

Amygdala PKC epsilon regulates corticotrophin releasing factor, anxiety-like behavior and alcohol consumption

HMB Lesscher[1,2], JK Deitchman[1], J Connolly[1], T McMahon[1], RO Messing[1]

PKCepsilon (-/-) mice show reduced alcohol self-administration and reduced anxiety-like behavior. Neuropeptides in the amygdala, particularly corticotrophin releasing factor (CRF), have been implicated in anxiety, including withdrawal-induced anxiety, and alcohol consumption. The goal of this study was to determine the role of PKCepsilon in the amygdala in these behaviors.

Amygdala CRF levels and mRNA abundance were reduced by 50% in PKCepsilon (-/-) mice and microinjection of 100 ng CRF into the amygdala increased anxiety-like behavior of PKCepsilon (-/-) mice to wild-type levels. By lentiviral expression of shRNA against PKCε, we decreased PKCε in the amygdala by 60%. Bilateral knockdown of PKCε in the amygdala decreased anxiety-like behavior in wild type mice. To explore the role of amygdala PKCepsilon in alcohol consumption we used a limited access alcohol consumption paradigm, a two-bottle choice task with access to water and 15% alcohol in a daily 2 hour session. Local knockdown of PKCepsilon in the amygdala reduced alcohol consumption in this paradigm, particularly after multiple daily drinking sessions. The results suggest that PKCepsilon regulates CRF expression in the amygdala and controls anxiety and alcohol consumption in mice.

[1] Ernest Gallo Research Center at UCSF, Emeryville, CA 94608, USA
[2] Rudolf Magnus Institute of Neuroscience, UMCU, Utrecht, THE NETHERLANDS
Supported by EC, Marie Curie Fellowship MOIF-CT-2004-002812 to H.M.B. Lesscher and Dept. of the US Army, DAMD 17-03-1-0058 to R.O. Messing.

7.00 pm: Dinner

Wednesday, May 23

8 am: Breakfast

8.45 – 10.45 am: Symposium 3

Behavioural phenotyping for alterations in brain function

Chair Sabine Hölter-Koch

8.45 am: Katharine Webb

Identification of mutations affecting amphetamine-induced reward in zebrafish

K Webb, J Ninkovic, W Norton, L Bally-Cuif
Addiction is a complex maladaptive behavior affecting millions of people worldwide. The rewarding properties of drugs of abuse are central for the development of addictive behavior and are most commonly measured by means of the conditioned place preference (CPP) paradigm. Katharine Webb will report here on the development of a reliable CPP test in adult zebrafish, its validation in a heterozygote background for the function of acetylcholinesterase (ache/+ zebrafish mutants), and its use in a genetic ENU screen aiming to the recovery of mutants affected in the reward process. To date, we isolated 13 dominant mutations leading to decreasing or abolishing amphetamine-induced CPP, and we are in the process of mapping these mutations.

GSF-National Research Center for Environment and Health, Institute of Developmental Genetics, Department Zebrafish Neurogenetics, GERMANY
Funding Support: EU Integrated Project ZF-Models No. LSHC-CT-2003-503466.

9.10 am: Pat Nolan

Cloning and characterisation of novel mouse circadian mutants in ENU screens

S Godinho[1], A Barnard[1], E Maywood[2], M Hastings[2], P. Nolan[1]

Mice as well as humans maintain a large number of physiological variables under constant control of an internal clock. The predominant rhythm adopted is circadian (~24-hours). Humans and mice keep under circadian control the regulation of diverse processes such as the sleep-wake cycle, locomotor activity, temperature regulation, metabolism, water/food intake and levels of circulating hormones. Disturbances in circadian parameters have been associated with a number of psychiatric and neurological disorders in humans including seasonal affective disorder, bipolar disorder and neurodegenerative disorders. Elucidation of the molecular basis of circadian rhythms has progressed dramatically over the past decade. Nevertheless, many molecular components remain to be identified in order to explain how the internal clock, residing within the suprachiasmatic nucleus of the hypothalamus, interacts with the environment and how it conveys its rhythm to oscillators in other tissues and brain regions. We have been conducting chemical mutagenesis screens in mice for mutations affecting circadian parameters. Using this approach we have uncovered a number of novel loci affecting circadian homeostasis. We will present details of the screens used, describe the mutant phenotypes identified and, finally, introduce the novel molecular insights into circadian homeostasis that have been uncovered by cloning the mutant genes.

[1]MRC Mammalian Genetics Unit, Harwell, Oxfordshire, and [2]MRC Laboratory of Molecular Biology, Neurobiology Division, Cambridge, UK
This work was supported by the MRC, BBSRC and by the 6th Framework Project EUCLOCK (No. 018741).

9.35 am: Silvia Mandillo

Mice lacking the parkin associated receptor GPR37 show behavioral abnormalities linked to dopaminergic system alterations

S Mandillo, E Golini, D Marazziti, C Di Pietro, E Meomartini, E Minicocci, R Matteoni, G Tocchini-Valentini

GPR37 is an orphan G-protein coupled receptor highly expressed in the mammalian central nervous system. It has been shown to be a substrate of parkin and to be involved in the pathogenesis of Parkinson's disease (PD). Gpr37-/- mice are viable and fertile, their gross brain morphology is normal, however they show alteration in spontaneous and amphetamine-induced locomotor activity, deficits in rotarod performance, reduced levels of striatal dopamine content, and resistance to MPTP-induced neurodegeneration (Marazziti et al., PNAS, 2004). Additionally Gpr37-/- mice display a reduced locomotor response to cocaine compared to their wild-type littermates and also a reduced cataleptic response to the administration of D1 and D2 receptor antagonists. We hypothesize these abnormalities to be related to the recently demonstrated interaction of GPR37 with the dopamine transporter (DAT). The study of this interaction could help in elucidating some of the mechanisms underlying PD and drug addiction.

CNR-National Research Council, Institute of Cell Biology, ITALY
Funding Support: Italian Ministry of Research projects (FIRB 2001, 2005, 2006; PS-PNR 2005-2007); European Framework Programme projects (EUMODIC, MUGEN and EURASNET).

10.00 am: Jan Deussing

CRH overexpression in the brain exerts antidepressant-like effects

A Lu[1], MA Steiner[1], AM Vogl[1], GK Stalla[1], F Holsboer[1], CT Wotjak[1], W Wurst[2], JM Deussing[1]

The implication of corticotropin-releasing hormone (CRH) in the pathophysiology of affective disorders has stimulated the interest in animal models of CRH hyperactivity. Therefore, different CRH overexpressing mice have been created, whose usability, however, was confounded by severe hypercortisolemia developing from unrestricted and exceeding CRH expression. To circumvent these problems, we created a highly flexible gain-of-function mouse model combining the Cre/loxP system with the ubiquitously expressed ROSA26 locus. This enabled us to dose-dependently overexpress CRH, restricted to the central nervous system (CNS). Thus, we avoided to affect basal emotionality and basal hypothalamic-pituitary-adrenocortical (HPA) axis activity. Under stressful conditions, however, CRH overexpression caused CRH receptor type 1 (CRHR-1) antagonist reversible alterations of stress coping behavior and HPA-axis hyperreactivity. Activating the endogenous CRH system by preceding stress exposure in wild-type mice, evoked similar alterations in stress coping behavior that could not be induced in CRHR-1 knockout mice. This demonstrates the value of conditional CRH overexpressing mice as an animal model for understanding the role of CRH in the transition from physiological to pathological stress adaptation.

[1]Max Planck Institute of Psychiatry, Munich, and [2]Institute of Developmental Genetics, GSF Research Center, Neuherberg/Munich, GERMANY

10.25 am: General discussion

10.45 am: Coffee

11.15 am: Distinguished Awardee lecture: Robert Williams

introduced by Daniel Goldowitz

Systems Genetics of Brain Structure and Function: A jigsaw puzzle

RW Williams

The process of understanding complex systems, such as the structure of the brain or the genetic modulation of behavior, bears similarities to the process of solving a jigsaw puzzle. The first step is mechanical and unrewarding--we flip pieces over to their correct side and pile up pieces that define edges and corners. The puzzle in our case has more than 27,000 colorful gene pieces. The second step is somewhat more interesting: blocks of particular colors and shapes are aggregated and subdivided. These are the gene ontologies and other metrics of sequence and functional similarity. Clever tactics and strategies are used to define and refine groups, depending on other sources of information. The third and longest step involves an interminable series of experiments to combine pieces that appear--often falsely--to have roughly the right shapes and colors.

Successful two-piece linkages of the type we have studied for the last 50 years (one gene variant mated to a single phenotype) are accumulated progressively. These doublets are expanded into clusters of three, four, and more multigenic pieces that begin to provide some vague ideas about small parts of the picture. Step 3 transitions surprisingly abruptly to a more exciting exponential fourth phase. Clusters of pieces are reoriented and eventually connected in ways that lead to minor and major epiphanies. During this process, brain reward systems spritz neurons in nucleus accumbens and elsewhere with well modulated doses of dopamine and other neurotransmitters. This mirrors our own scientific satisfaction that we are beginning to understand the complex neurogenetics that underlies this whole puzzling brain reward system. Finally, the process of adding correct pieces into the puzzle becomes ridiculously easy. As our knowledge grows, there are far fewer degrees of freedom and far fewer errors. In this final fifth phase, we snap pieces in place almost without thinking. The enjoyment shifts from the action of solving the puzzle to understanding and appreciating the image of the puzzle: holistic systems integration. In the case of complex biological systems, we hope that this understanding translates in some way to longer, more rewarding, and healthier lives.

Many of us have spent much of our careers diligently flipping over or fondling single pieces of the puzzle. If we have had luck and worked hard, we may even have had the opportunity to snap a few pieces together. Some of us are building analytic/synthetic tools--the tables, chairs, lights, and eyes--that are the necessary infrastructure to solve these complex puzzles; GeneNetwork being one example. As a community we are now in the middle of the exponential phase. To ruin the metaphor; we are now simultaneously and collaboratively working on steps 1, 2, 3, and 4. Pieces are being discovered, snapped together, and globally positioned into a large vista at an accelerating rate. It is an exciting time to be solving puzzles. The selective forces that have shaped the evolution of these sequences, but have also in some instances provided insights into the mechanics of the clock. I will describe some

examples of this type of work by focusing on the period and timeless genes in insects.

Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, Tennessee, USA

12.15 pm: Lunch

1.45 – 3.45 pm: Symposium 4

COMT Val158Met: a uniquely human balanced polymorphism

Chair Mary-Anne Enoch, National Institute on Alcohol Abuse and Alcoholism, USA

1.45 pm: Mary-Anne Enoch

The influence of COMT genotype on anxiety and addiction

M-A Enoch, J Waheed, C Harris, KV White, B Albaugh, D Goldman

Val158Met is a uniquely human, functional polymorphism in the catechol-O-methyltransferase (COMT) gene that is abundant across many different ethnic groups suggesting maintenance by balanced selective forces. COMT plays a major role in the metabolism of CNS dopamine and norepinephrine. We genotyped individuals from two community samples from very different backgrounds: 350 Plains American Indians and 210 U.S. Caucasians. All participants were DSM-III-R psychiatrically interviewed. They completed the TPQ and WAIS-R and underwent EEGs. We found that the variant low activity Met158 allele was linked with greater severity of pathological anxiety, and in both Caucasian and Plains Indian women the Met/Met genotype was associated with a more anxious, cautious personality together with an EEG intermediate phenotype for anxiety. On the other hand, in the Plains Indians the Met158 allele was associated with increased years of education, superior executive functioning (more so in non-al coholics than in alcoholics) and was protective against alcoholism and smoking (women only). The results of our study, put together with those of others, show that the variant low activity Met158 allele underlies increased emotional sensitivity together with superior cognitive skills but on the downside, with increased stress-related pathology. In contrast the ancestral Val158 allele is associated with increased stress resiliency but also with poorer cognitive abilities that are characteristic of diseases such as schizophrenia and drug addiction. In addition, both the Val158 and Met158 alleles can be risk factors for alcoholism in different drinking environments. Finally, there appear to be sexually dimorphic effects in the relationship between COMT Val158Met and anxiety.

Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, Maryland USA
Funding Support: Office of Research on Minority Health.

2.10 pm: Andreas Heinz

COMT genotype effects on central processing of affective stimuli

A Heinz, M Smolka, D Müller, J Wrase

In behavioral genetics, additive gene-gene effects are often assumed but difficult to test due to the small explained behavioral variance. Processing of unpleasant stimuli in the amygdalae has been associated with a functional polymorphism (val158-met) in the catechol-O-methyltransferase (COMT) gene and independently with a functional polymorphism in the regulatory region of the serotonin transporter (5-HTT) gene. 5-HTT function may also be affected by a recently detected A/G exchange in the long allele (insertion) of the 5-HTT regulatory region. We tested whether these three genotype effects are additive and explain a substantial part of the variance in amygdala activation elicited by unpleasant cues. Genotype effects on central processing of standardized affective visual stimuli (unpleasant and neutral) were assessed in 48 healthy men with functional magnetic resonance imaging. In individuals with more COMT met158 alleles and with more s or lA alleles of the 5-HTT regulatory region, aversive stimuli elicited greater neuronal activity in the bilateral amygdala and hippocampus. COMT val158-met and 5-HTT genotype were additively associated with increased processing of aversive stimuli in the amygdala, indicating that functional brain imaging may be used to assess the interaction of multiple genotypes on neuronal activation.

Department of Psychiatry, Charité University, Berlin GERMANY
Funding Support: DFG He 2597/7-3.

2.35 pm: Elizabeth Tunbridge

The role of COMT in prefrontal function and its relevance to schizophrenia

EM Tunbridge

The catechol-o-methyltransferase (COMT) enzyme metabolises catecholamines, including dopamine, which is critical for regulating prefrontal cortex (PFC) function. The human COMT gene contains a polymorphism (Val158Met), which alters enzyme activity and is associated with PFC function. In addition, COMT may be genetically associated with schizophrenia. This presentation will review preclinical data linking COMT, dopamine function and cognition, as well as discussing emerging complexities to the biology of COMT. The importance of COMT for modulating PFC neurochemistry and behaviour was investigated in the rat using tolcapone, a brain-penetrant COMT inhibitor. Administration of tolcapone had no effect on basal levels of dopamine or noradrenaline in medial PFC (mPFC), measured using in vivo microdialysis, but significantly potentiated stimulated dopamine, but not noradrenaline, efflux. Consistent with these findings, and with data linking the human Val158Met polymorphism with cognition, tolcapone specifically improved performance on a task known to depend on dopamine function and the PFC. In addition, the presence of COMT variants was investigated in human PFC. This revealed both mRNA and protein COMT variants, which could potentially enhance or confound genetic associations with cognition and psychiatric disorders. These data demonstrate that COMT is important for modulating dopamine levels in the PFC, and for modulating PFC-dependent behaviour. These data are strikingly similar to findings linking the human COMT Val158Met polymorphism and suggest that COMT might be a viable drug target for treating the cognitive dysfunction suffered by patients with schizophrenia. In addition, our discovery of novel variants suggests an additional level of complexity to the biology of COMT. Investigation of the functional impact of these variants and their expression in psychiatric disorders and associated with COMT polymorphisms is ongoing; however, they may be important modulators of COMT's function in the brain.

Department of Psychiatry, University of Oxford, Oxford, UK

3.00 pm: Ben J Baig, University

Relationship of COMT variants to brain structure, function and risk of psychosis

AM McIntosh, BJ Baig, J Hall, D Job, HC Whalley, GKS Lymer, TWJ Moorhead, DGC Owens, P Miller, D Porteous, SM Lawrie, EC Johnstone

Introduction: There is growing evidence that the gene COMT may be involved in the aetiopathogenesis of schizophrenia. This study sought to clarify the effects of the COMT variant on brain structure, function and risk of developing schizophrenia in a well-characterised cohort of individuals at high risk of schizophrenia for familial reasons. Methods: In a sample of people at high risk of schizophrenia, the risk of progression to schizophrenia associated with the COMT Val allele was estimated. The relationship of the Val allele to brain structure and function was then investigated using structural and functional MRI data collected on the high-risk subjects before their disease outcome was known. Results: The COMT Val allele was shown to increase the risk of schizophrenia in this cohort in a dose-dependent manner. Subjects with the COMT Val allele had reduced grey matter in anterior cingulate cortex. In addition, there was evidence of increased activation in lateral prefrontal cortex, anterior and posterior cingulate with increasing sentence difficulty in those with the COMT Val allele despite a similar level of performance. Discussion: The COMT Val allele is associated with an increased risk of schizophrenia in subjects at increased familial risk. It has demonstrable effects on frontal brain structure and function. These patterns of altered brain structure and function have previously been associated with schizophrenia in this and other samples.

University of Edinburgh, UK
Funding Support: MRC.

3.25 pm: General discussion

3.45 pm: Break

4.15 – 6.15 pm: Poster session

Levodopa treatment rescues memory deficits in transgenic mice modelling Alzheimer's disease

O Ambrée[1], H Richter[1], L Lewejohann[1], K Keyvani[2], W Paulus[2], WR Schäbitz[3], N Sachser[1]

Dopamine plays an important role in learning and memory processes. A deficit of this neurotransmitter as it is apparent in Alzheimer's disease may contribute to cognitive decline, a major symptom of Alzheimer patients. The aim of this study was to elucidate if stimulation of the dopaminergic system is able able to improve cognitive function in a transgenic mouse model of Alzheimer's disease.

Male mice of the TgCRND8 line carrying a double mutated form of human APPswe&ind and their wild type littermates were treated either with levodopa or vehicle (saline) and tested in an object-recognition task and the Barnes maze.

Concerning object-recognition memory wild type mice of both treatment groups performed equally well. Transgenic animals, however, showed a significant object recognition only when treated with levodopa while vehicle treated transgenics did not discriminate between the familiar and the novel object. In the Barnes maze both wild type groups performed similarly well while vehicle treated transgenic animals had a significantly longer latency and ran a significantly longer path until they found the escape hole. In transgenic animals levodopa treatment led to a significantly better acquisition of spatial memory compared to vehicle treated transgenic mice.

In summary, deficits in object-recognition memory and spatial learning of TgCRND8 mice could be ameliorated by levodopa treatment. We hypothesise that the dopaminergic system is an important target in order to counteract cognitive decline in senile dementia.

[1]Department of Behavioural Biology, [2]Institute of Neuropathology and [3]Institute of Neurology, University of Münster, GERMANY
This work was supported by BMBF grant 01GW0520.

Fmr1 KO mice as a possible model for autism

M Bernardet, W Crusio

Although autism was first described in 1943, little is still known about its etiology and that of related developmental disorders. Work with human patients has provided many data on neuropathological and cognitive symptoms but our understanding of the functional defects at the cellular level and how they come about remains sketchy. To improve this situation, autism research is in need of valid animal models. However, despite a strong hereditary component, attempts to identify genes have generally failed, suggesting that many different genes are involved. As a high proportion of patients suffering from the Fragile X Syndrome show many autistic symptoms, a mouse model for this disorder could potentially also serve as a model for autism. The *Fmr1* KO mouse is a valid model of the Fragile X Syndrome and many data on its behavioral and sensory-motor characteristics have already been gathered. We present an overview of literature reporting autistic features in this candidate model. Although more work is needed, we feel that it is highly likely that *Fmr1* KO mice constitute a valid model for autism.

Centre de Neurosciences Intégratives et Cognitives, Université de Bordeaux I, CNRS
UMR 5228, 33405 Talence, FRANCE

This work was supported by grants from the March of Dimes (12-FY05-1198), Conseil
Régional d'Aquitaine, CNRS, and the University of Bordeaux I to WEC.

The long-term effects of cannabis use in early adolescence; an investigation in mouse models of cognitive function

E Binder, C Fernandes, LC Schalkwyk, DA Collier

Although it is widely held that cannabis is a safe drug, there are potential side-effects to its use, including cognitive impairment, adverse psychological reactions such as anxiety and paranoia, and an increased risk of developing psychosis. It is clear from studies of psychosis that some individuals are more vulnerable to its effects than others, and that the timing of use may amplify its effect. We investigated the long-term effect of continuous δ-THC (delta-9-Tetrahydrocannabinol) use in two inbred strains of mice; C57BL/6J and DBA/2J. These strains are known to differ in their innate anxiety-related behaviour and cognitive abilities. Two different time windows were chosen to administer the drug: one group was treated from PND 7 – 21 while another group of mice were treated from day 30-44. δ-THC was solved in pure sesame oil and 20 mg/kg bodyweight were administered once a day orally for 14 days. At the adult stage age of 56 days the mice were tested first in an object recognition task and one week later in a delayed match to place (DMP) version of the Morris water maze. These two behavioural paradigms assess different memory systems in the brain. The Object Recognition task is a non-spatial task and not hippocampus-dependent. The DMP has been proposed as a model of working/episodic-like memory and has been shown to involve the hippocampus and NMDA receptor activation. The results show that the drug treatment induced a difference regarding the cognitive performance of the animals depending on the strain and time of treatment.

Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, UK

A behavioural analysis of the Coloboma mutant mouse

RE Brown, ME Keenan, RK Gunn

The Coloboma mutant mouse (C3H/HeSnJ-cm) has been proposed as an animal model of attention-deficit hyperactivity disorder (ADHD) due to excessive locomotion in an open field, yet few behavioural studies have looked at other behavioural measures in these mice. In our study, we looked at male and female Coloboma mice and their littermate controls (C3H/HeSnJ) in two different sizes of open field, pre-pulse inhibition, acoustic startle, Rotarod, balance beam, spontaneous alternation in a Y-maze, conditioned odour preference, rewarded alternation in the T-maze, tail-flick test and hot-plate test. We found excessive hyperactivity and a lack of habituation in these mice, but no deficits in cognition or in pre-pulse inhibition. Results from the Rotarod and balance beam tasks indicate that Coloboma mice have severe motor coordination and balance problems compared to their littermates, suggesting that Coloboma mice may be a model of ataxia rather than ADHD.

Department of Psychology, Dalhousie University, CANADA
Funding Support: NSERC of Canada.

Using Chromosome Substitution Strains to identify gene-environment interactions for behavioural traits in mouse

C Fernandes, E Pjetri, H Oppelaar, MJH Kas

Social environment plays an important role in the development of behavior. For example, absence or loss of social support, have been linked to both the onset and relapse of depression (Paykel, 1994) and social support may protect against stress-related disorders (deVries et al, 2003). There is growing evidence for gender differences in response to such support (Glynn et al, 1999), which may be a reflection of the different coping strategies employed by men and woman in response to stress (Taylor et al, 2000). Social housing has been proposed as a rodent model of social support (Westenbroek et al, 2003), with social instability affecting females more than males (Haller et al, 1999). Although adverse life events and the development of behavioural disorders are clearly related, the behavioral and genetic mechanisms underlying this relationship are not fully understood. In this study we have used this mouse model of social support in combination with the C57BL/6J-Chr #^{A}/NaJ chromosome substitution strains (CSS). CSS offer several advantages for dissecting complex traits and are a powerful tool to identify genes for behaviour in mouse (Singer et al., 2004). The effect of manipulating social environment across different genetic backgrounds was studied using females from 10 lines of the C57BL/6J-Chr #^{A}/NaJ CSS panel. Mice were run through a large battery of tests to assess behaviors ranging from anxiety to cognitive ability. Results from the anxiety battery will be presented.

Department of Pharmacology & Anatomy, Behavioural Genomics Section, Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, THE NETHERLANDS
This work was supported by a Marie Curie Intra European Fellowship to C. Fernandes and a UMC Utrecht foreign exchange program grant to M.J.H. Kas.

Wild mouse Open Field behavior is embedded within the multidimensional data space spanned by laboratory inbred strains

E Fonio[1], Y Benjamini[2], A Sakov[2], I Golani[1]

The vast majority of studies on mouse behavior are performed on laboratory mouse strains (Mus 'laboratorius'), while studies of wild mouse behavior are relatively rare. An interesting question is the relationship between the phenotypes of Mus 'laboratorius' and the phenotypes of their wild ancestors. It is commonly believed, often in the absence of hard evidence, that the behavior of wild mice exceeds by far, in terms of repertoire richness, magnitude of variables, and variability of behavioral measures the behavior of the classical inbred strains. Having phenotyped the Open Field behavior (OF) of 8 of the commonly used laboratory inbred strains, two wild-derived strains, and a group of first-generation-in-captivity local wild mice (Mus musculus domesticus), we show that contrary to common belief, wild mouse OF behavior is moderate, both in terms of endpoint values and in terms of their variability, being embedded within the multidimensional data space spanned by laboratory inbred strains. The implication could be that whereas natural selection favours moderate locomotor behavior in wild mice, the inbreeding process tends to generate in mice, in some of the features, extreme and more variable behavior.

[1]Department of Zoology, George S. Wise Faculty of Life Sciences, and
[2]Department of Statistics and Operations Research, The Sackler Faculty of Exact Sciences, Tel Aviv University, ISRAEL
This study was supported by the Adams Super Center for Brain Studies, Tel-Aviv University, Israel.

A model to evaluate the vulnerability to eating disorders in female mice

L Garbugino, F D'Amato, A Moles

Binge eating (BED) is an eating disorder whose pathophysiological mechanisms are still not clear. There is evidence of the existence of different polymorphisms in genes of the serotonin (5HT) system in patients exhibiting binge eating behavior.

Mice are largely used to identify the genetic basis and heritability of normal and pathological behaviors. In humans, dieting strongly predicts stress-induced overeating. Basing on this evidence, Hagan and coll. developed an animal model for the study of BED using female rats, which derives from the hypothesis that caloric restriction and stress interact to induce binge eating. The model consists of exposing the animals to 3 consecutive cycles, each divided in 3 phases : food restriction, refeeding and exposition to high palatable food after stress (electric shock).

The study was aimed at verifying if this model can be applied also to mice. Our results showed that binge eating was induced in adult female mice that were subjected to both food restriction and stress even after the first cycle of restrain-refeeding. Therefore this model can be suitable also to evaluate vulnerability to binge eating disorder in different strains of mice.

CERC-CNR of Rome Institute of Neuroscience, ITALY

Gene to phenotype networks for alcohol and drug addiction

D Goldowitz, T Ansah, C Blaha, M Cook, K Hamre, G Mittleman, E Chesler

Drug abuse and addiction are complex phenotypes. Typical of many human diseases, they are influenced by multiple genetic and environmental factors. Susceptibility to addiction is co-morbid with other behavioral disorders, which is evidence that the same genetic influences may be acting to affect multiple phenotypes. The purpose of this project is to systematically identify genes and gene networks that modulate pleiotropic responses to abused substances, behavioral variation, and susceptibility to abuse. This application exploits the unique mapping properties of RI strains, a new, high power expanded set of BXD RI lines, advanced bioinformatics tools, extensive databases present in WebQTL, and the expertise of the Tennessee Mouse Genome Consortium high-throughput phenotyping resource to systematically identify upstream genes and molecular networks that ultimately modulate downstream pleiotropic drug and alcohol phenotypes. It is thought that data resources generated by this project will dramatically reduce the amount of phenotyping one needs to perform to discover the effects of any novel gene specific mutation. Candidate genes will be validated using publicly available mouse mutant resources. This will be invaluable for the development of realistic complex disease models and will provide data resources to suggest cost effective targeted phenotyping strategies for large scale single gene mutation efforts such as those proposed by the Comprehensive Knockout Mouse Project Consortium. More broadly, we will be able to identify the specific genetic basis of the pleiotropic and polygenic effects of genetic polymorphisms on drug abuse, addiction, and individual differences in brain and behavior.

University of Tennessee Health Science Center, USA
Funding Support: R01DA020677 and U01AA016662.

Susceptibility genes for sporadic febrile seizures

E Hessel, K van Gassen, I Wolterink, M Kas, P de Graan

Febrile seizures (FS), which are the most common seizure types in childhood, affect about five percent of children. Complex FS may be involved in the development of temporal lobe epilepsy (TLE) during adulthood, but the underlying mechanism of FS remains unknown. Recent association, family and twin studies indicate that genetic background is important in FS. The aim of this study is to identify FS susceptibility genes and cascades using a panel of mouse chromosome substitution strains (CSS).
Complex febrile seizures were induced in the host (C57) and donor (A/J) strain of the panel by exposure of mice to a 50 ^{0}C air stream for 900s at postnatal day 14. During hyperthermia mouse behavioral repertoire and body temperature were continuously monitored with Ethovisionth. In this paradigm the C57 mice are more susceptible to hyperthermia-induced seizures than the A/J mice. We have screened the full CSS panel and identified four strains with an AJ phenotype. We have started to backcross these strains to map the quantitative trait loci (QTL's) on the respective chromosome.

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Body: Novel object exploration in mice: the complex nature of habituation

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Here we report the influence of familiarization on object exploration in C57BL/6J and DBA/2J mice. Familiarization is an initial exposure to the open field prior to object presentation. Our protocol consists of four 6-min trials (Trial 1-no objects; Trials 2,3-two objects placed in the open field; Trial 4-one object is replaced with a novel object). In this study, additional groups of mice received a 24-min pre-exposure to one of two different open fields (without objects) the day before testing and a group that experienced the open field and objects together for the first time (no familiarization and no Trial 1). As expected, DBA mice pre-exposed to the open field exhibited the greatest amount of object exploration. In contrast, C57 mice given the longest pre-exposure to the open field showed the least interactions with the objects. The greatest amount of object exploration was observed in C57 mice given no pre-exposure to the open field. Moreover, C57 mice exhibited reduced inner square crosses and increased stretch-attends when the objects were presented in a more familiar context; behaviors consistent with an increase in anxiety. These results suggest that pre-exposure affects the figure/ground relationship between the objects and the open field. Superior performance in DBA mice was obtained by pre-exposure to any apparatus prior to object presentation (habituation to the overall "experiment"). In contrast, the behavior of C57 mice appears to be contextually bound (habituation is specific to an "environment"); a change in exploratory behavior was observed when local conditions were altered. Therefore, novelty-induced behaviors can be elicited by an unfamiliar context, stimuli, or changes in a familiar context, with these results dependent on the organism's genotype.

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Genetic analysis of hippocampal iron, copper, and zinc content in 30 BXD Recombinant Inbred Strains of mice

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Trace amounts of Fe, Cu, and Zn are essential for optimal hippocampal functioning, but these metals require strict regulation. To investigate the genetic components of this regulation, we performed a QTL analysis of hippocampal Fe, Cu, and Zn concentrations in the BXD strain panel. A colony of 30 BXD mouse strains was fed a standard laboratory diet (n.m.t. 240 ppm Fe, 18 ppm Cu, and 124 ppm Zn) from weaning to 120 days of age. At 120 d, the hippocampus was removed for quantification of Fe, Cu, and Zn by ICP-MS. Results revealed genetically-based variation in hippocampal Fe, Cu, and Zn content; i.e. strain was a significant main effect ($p<0.001$) on each metal in a two-factor ANOVA (strain, sex). Among 30 strains, the range of variation in the hippocampal concentrations of these metals was 3-fold for Cu (2.8-10.3 ugCu/g), approximately 2-fold for Fe (11.0-19.3 ugFe/g), and 1.5 fold for Zn (15.1-24.9 ugZn/g). Mean Fe, Cu, and Zn concentrations were positively correlated in the hippocampus, suggesting coregulation. The distribution of mean Fe, Cu, and Zn concentrations across the strain panel was continuous, suggesting that trace metal regulation is a complex phenotype influenced by multiple genes and gene-environment interactions. Heritability estimates for Fe, Cu, and Zn were 0.25, 0.29, and 0.15, respectively. QTL analysis revealed one significant QTL (LOD 4.69) on chromosome 14 (38.7 Mb) associated with Cu in the male and female hippocampus and one QTL on chromosome 9 (40.2 Mb) associated with both Cu and Zn in the male hippocampus. Comparisons of this data with previous data on the BXD panel showed that hippocampal concentrations of these metals are positively correlated with hemoglobin, hematocrit, TIBC, and performance in the Morris Water Maze (a hippocampus-dependent task), but are not correlated with metal content in other brain regions.

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Evaluation of the alpha 3 subunit of the nicotinic acetylcholine receptor as a candidate gene for ethanol-induced locomotor activation

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Alcohol and nicotine are commonly co-abused drugs. Genetic correlations have been reported between the responses to these drugs in both human and animal models, suggesting that common genes may underlie the response to both drugs. There is evidence that a gene resides on mouse chromosome 9 that accounts for some of the phenotypic variance in the acute locomotor response to ethanol. One gene that resides in this area is the alpha 3 subunit of the nicotinic acetylcholine receptor (Chrna3). Since acetylcholine receptors are important in the behavioral response to nicotine, we used pharmacology, gene expression, and protein expression assays to determine if this gene is important in ethanol-induced locomotor stimulation. We first confirmed the presence of a gene on chromosome 9 using DBA/2J mice and congenic mice that possessed C57BL/6J alleles from 9 – 61 cM on a DBA/2J background. Mecamylamine, a nonspecific nicotinic acetylcholine antagonist, was able to block the acute stimulant response to ethanol. Congenic mice expressed significantly greater Chrna3 mRNA compared to the DBA/2J control strain, but this did not appear to translate into differences in the amount of alpha 3 containing nicotinic receptors as measured by receptor binding. These data provide evidence for the involvement of nicotinic acetylcholine receptors in ethanol-induced stimulation, but it remains unclear whether the Chrna3 gene is the quantitative trait gene on chromosome 9.

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Genetic dissection of behavioral traits related to eating disorders using chromosome substitution strains of mice

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Eating disorders are complex psychiatric disorders in which patients display a variety of behavioral traits, including obsessionality, increased anxiety, hyperactivity and altered appetitive motivation. These traits are also seen in other psychiatric disorders, such as in obsessive-compulsive disorders and depression, which are often co-morbid with eating disorders. Family and twin studies have revealed that genetic factors play a major role in eating disorders, but attempts to find susceptibility genes through linkage and association studies have been largely unsuccessful. Thus, novel approaches are required to deal with both phenotypic and genetic heterogeneity and with gene-environment interactions underlying these disorders. Recent studies have shown that genetic variation associated with psychiatric disorders affect analogous neuro-anatomical and behavioral traits in mice and men, demonstrating that mouse models can contribute to systematic searches for genetic determinants of psychiatric disorders (Chen et al., 2006). Therefore, to identify novel genetic loci regulating mouse behavioral traits related to eating disorders, new and sensitive mouse genetic mapping and inbred populations will be exposed to a comprehensive animal model of eating disorder traits (Kas et al., 2003; Gelegen et al., 2007). In an initial screen using this method, specific behavioral eating disorder traits were linked to mouse chromosomes that are syntenic with human linkage regions for anorexia nervosa and obsessive-compulsive disorders. These findings offer a great opportunity to translate essential behavioral traits in animals to human eating disorders and to further understand the molecular genetic mechanisms underlying these traits.

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Insulin regulation of ethanol-induced locomotor stimulation in *Drosophila melanogaster*

CL Kliethermes, U Heberlein

Insulin signaling has classically been studied for its role in glucose homeostasis and the regulation of gene transcription, along with associated effects on diabetic disease states and longevity. More recently, a direct role for brain insulin receptor signaling in the modulation of motivated and drug-related behaviors has become apparent. We used Drosophila melanogaster to study the role of insulin signaling in the modulation of ethanol-induced locomotor stimulation. Flies heterozygous for one of three mutated insulin receptor alleles were first compared to wild-types at a moderate, stimulatory dose of ethanol. All three mutants showed markedly higher ethanol-induced stimulation compared to wild-types, as did flies heterozygous for a mutation in chico-1, the Drosophila homolog of mammalian insulin receptor substrate, and p60, an inhibitor of insulin signaling. Using the UAS/GAL4 binary expression system, we next over-expressed several constitutively active forms of the insulin receptor in neurons and observed an unexpected, but similar increase in ethanol-induced stimulation relative to control strains. Neural over-expression of two different dominant negative forms of the insulin receptor was without effect. To determine which of the several Drosophila insulin-like peptides (dILPS) might mediate ethanol-induced locomotor stimulation, we over-expressed four different dILPS in neurosecretory cells. Over-expression of each dILP resulted in a modest to large increase in ethanol-induced stimulation, indicating that a single insulin-like peptide does not mediate insulin's effects on ethanol stimulated locomotion. Since both genetic inhibition and activation of the insulin signaling pathway resulted in enhanced ethanol-induced locomotor stimulation, these results suggest that any perturbation of insulin signaling might function to enhance sensitivity to ethanol's locomotor stimulatory effects.

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Systematic analysis of behavioral traits in a series of consomic mouse strains established from C57BL/6J and wild-derived MSM

T Koide, A Ishii, A Nishi, T Shiroishi, A Takahashi

Much of the genetic variation that underlies most behavioral traits is complex and is regulated by loci that have quantitative effect on the phenotype. We aimed to reveal those genetic mechanisms underlying individual differences of behavior by using consomic strains (CSSs) of mouse. We have previously shown that laboratory strain C57BL/6J and wild-derived strain MSM have great differences in many behavioral traits. A series of CSSs are established by replacing any one chromosome of C57BL/6J with a corresponding chromosome of MSM. By examining a set of CSSs, we successfully mapped the chromosomes that have a locus or loci affecting several behavioral phenotypes: home-cage activity, emotionality-related behavior (two consecutive open-field tests, light/dark box test, and elevated plus-maze test), pain sensitivity (hot-plate test and tail-flick test), and social interaction behavior. For open-field test, we performed ethological observation for 12 behavioral items (e.g. stretching, rearing, jumping and so on) to understand their behavior in detail. The result indicated that most behavioral phenotypes had contributions from a number of chromosomes, and each chromosome had prominently large effect on the phenotype. In addition, some of those chromosomes had sex-dependent effects. Genetic correlations between home-cage activity and other behaviors showed modest correlation between spontaneous activity and open-field activity but not other measurements. Principal component analysis was performed to reveal the relations between emotionality-related behaviors, and we extracted six factors underlying those behaviors. Analysis of CSSs allowed us to map several chromosomes associated with those six fundamental constructs that underlie emotionality-related behaviors. In contrast, only two CSSs, B6-6CMSM and B6-17MSM, exhibited significant increase of social contact compared to C57BL/6J. For the pain sensitivity tests, our result suggested hot-plate test and tail flick test possess some common genetic loci for pain perception and some test-specific genetic loci related to the reflex reaction.

National Institute of Genetics, JAPAN

Hormonal control of molecular mechanisms involved in learning and memory

HJ Krugers[1], L Conboy[2], O Wiegert[1], D Holman[3], JM Henley[3], C Sandi[2], M Joëls[1],CC Hoogenraad[4]

Emotionally arousing and stressful events are remembered well in general. These memories are facilitated by corticosteroid hormones, which are released during the stressful situation. One of the questions is how these hormones exert their effects.

AMPA receptors are critically involved in synaptic efficacy, plasticity and learning and memory processes. Electrophysiological data indicates that corticosteroid hormones, via activation of the glucocorticoid receptor increases AMPA receptor mediated synaptic transmission in ventral tegmental area and hippocampus. Accordingly, we will present evidence that these hormones induce trafficking of AMPA receptors to the membrane. These effects mediated via glucocorticoid receptor and involve a genomic action.

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Quantitative traits for the tail suspension test: automation, optimisation and BXD RI mapping

HV Lad, L Liu, JL Payá-Cano, C Fernandes, LC Schalkwyk

Immobility in the tail-suspension test (TST) is considered a model of despair in a stressful situation, and acute treatment with antidepressants reduces immobility. Inbred strains of mouse exhibit widely differing baseline levels of immobility in the TST and several Quantitative Trait Loci (QTLs) have been nominated. The labour of manual scoring and varying scoring criteria are problems in obtaining robust data and in comparing across different laboratories. Several studies have validated strain gauge and video analysis methods by comparison with manual scoring. We set out to find objective criteria for automated scoring parameters that maximise the biological information obtained, using a video tracking system on tapes of tail suspension tests of 24 lines of the BXD recombinant inbred panel and the progenitor strains C57BL/6J and DBA/2J. The maximum genetic effect size is captured using the highest time resolution and a low movement threshold. Dissecting the trait further by comparing genetic association of multiple measures reveals good evidence for loci involved in immobility on chromosomes 4 and 15. These are best seen when using a high threshold for mobility, despite the overall better heritability at the lower threshold. A second trial of the test has greater duration of immobility and a completely different genetic profile. Frequency of mobility is also an independent phenotype, with a distal chromosome 1 locus.

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Selection for high litter size in mice produces genetic changes in behaviour and haemoglobin expression in the brain

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We have used a combination of behavioural analysis, quantitative brain gene expression profiling, selective breeding and traditional strain crosses, to search for molecular mechanisms involved in anxiety-like traits in mice selected for high production traits during more than 120 generations.

We identified transcripts of haemoglobin genes with altered expression patterns inherited in an additive manner, which correlate with anxiety-like behavioural phenotypes. Our results show that selection for high litter size, which is often used by animal breeders, can result in unwanted inherited changes in anxiety-related traits. Moreover, these changes may be caused by an inherited altered expression of haemoglobin related genes. Although the possible involvement of haemoglobin genes in behaviour is surprising, the results are well in agreement with our previous experiments showing that selection for tameness modulates the expression of heme-related genes in silver foxes (Lindberg et al. Current Biology 2005 15: R915-6).

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Dissection of anxiety-related behavior and impulsivity in inbred strains of mice

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Behavioral phenotypes are complex traits that emerge from the concerted action of multiple genes. One approach to reduce this complexity is to dissect behavior using a series of behavioral tests, and mathematically extract dimensions underlying the behavior in these tests. The extracted dimensions can be used in a correlation analysis with molecular phenotypes, or in a genetic mapping study to identify the underlying genes. One potential obstacle in this approach is contribution of both genetic and environmental variation, which may influence the extracted dimensions to a different extent. In this study we assessed the performance of a panel of six inbred strains of mice on 43 measures, capturing the behavioral repertoire of mice ranging from anxiety-induced behavioral inhibition to reward-motivated impulsive behavior. Genetic correlations between phenotypes were calculated from strain means, and the environmental correlations from the individual performance normalized for strain. We observed that environmental variation induced strong correlations between phenotypes measured in the same test, resulting in extracted dimensions related to specific tests (so-called "test session factors"). By contrast, genetic variation induced correlations between phenotypes measured in different tests, resulting in the discrimination of 5 genetic dimensions of which 1 was related to impulsivity and at least 2 were indicative of different types of anxiety. We conclude that the use of mean performance of inbred strains, rather than the performance of individual mice, serves as valuable approach to
dissect complex phenotypes into underlying genetic dimensions.

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19. Nuclear calcium signaling is required for the formation of long term memory

CE Margulies, J Weislogel, H Bading, CM Schuster

Animals store information based on their individual experiences. Yet not all information is stored for long periods of time. Rather, it is the most intense events, or the most repeated information, that is encoded in long-term memory (LTM). However, the processes that “filter” information and convert it to LTM are not well understood. LTM is consolidated memory that depends on DNA transcription and RNA translation and therefore requires signaling from distal synapses to the cell nucleus. It is presently unclear which signals lead to these changes in transcription.

We have investigated the requirement and the role of nuclear calcium signaling in the formation of LTM using a classical olfactory learning and memory paradigm. We present experimental data using transgenic Drosophila which implicate nuclear calcium as a necessary signal for LTM formation. This signaling is required during acquisition of memory and – at least initially – is not required during consolidation of memory.

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20. Hunger-motivated learning in eight inbred strains of mice in two configurations of the tunnel maze

EM Munn[1], SR Prada[1], D Wahlsten[1,2]

The tunnel maze was used to assess learning motivated by hunger in eight inbred strains of mice (129S1/SvImJ, A/J, BALB/cByJ, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ, and SJL/J). This is a test of simple learning that can be performed by mice with retinal degeneration if they can recall what to do. Mice must learn to keep moving ahead and not to reverse direction. They can utilize their own odour trails to tell them which parts of the maze have already been visited. A slightly more complicated version of this maze is the Lashley III configuration which includes blind alleys. In this task, the mouse must learn not to reverse direction and not to enter blind alleys. Performance in the straight alley versus the Lashley III configuration of a 5-alley maze was compared over ten days of testing (2 trials/day). Most mice learned to traverse the maze in order to retrieve a food reward, but acquisition was more reliable with the Lashley III configuration. The mice made fewer errors (backtracks, blind alley entries) with repeated testing, so that the distance travelled and the latency to reach the goal were reduced. Although mice of all strains were successful in retrieving the food reward, strain differences were noted in the number and type of errors and in measures related to motor performance.

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21. Stress hormone effects on cognitive performance: an integrated behavioural and morphological analysis in mice

MS Oitzl[1,2], V Brinks[1], O Wiegert[2], H Krugers[2]

Corticosteroid stress hormones (cortisol, corticosterone) can facilitate as well as impair emotional and cognitive processes and neuronal plasticity. The goal of the project is to unravel the integration of these processes in relation to receptors in the brain that mediate the action of the corticosteroids. We demonstrate the differential effects of corticosteroids on (i) emotion and cognition using a fear conditioning task and (ii) molecular mechanisms involved in learning and memory, i.e. AMPA receptors. Behaviour: Male C57BL/6 mice were tested for fear conditioning (freezing) with or without corticosterone administration. Corticosterone before and after acquisition results in resistance to extinction for cue. Corticosterone and saline after acquisition increased memory for the context, while corticosterone also results in a better differentiation between context and cue retrieval. Morphology: Acute slices and primary cultures were incubated with corticosterone for 20 min a nd 3 hr respectively.

Corticosterone differentially changes the surface expression of GluR1 and GluR2 AMPA receptors. We concluded that increased stress hormones before or directly after fear conditioning, strengthens the memory for the negative event. There is no extinction of the fear response (freezing) to the stimulus that previously announced the punishment. Corticosterone enhances surface expression of GluR2 subunits. We hypothesize that this stabilizes synaptic strength, and makes it more difficult to encode novel information. With this data we are working towards a model for Post Traumatic Stress Disorder.

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22. Anxiety and ethanol withdrawal: elevated plus maze and other anxiety-related behaviors in Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice

SD Philibin[1,2], AJ Cameron[1,2], JC Crabbe [1,2,3]

Ethanol withdrawal produces physical and psychological symptoms. The psychological or negative affective symptoms are highly correlated with relapse in alcoholics. The neurobiological mechanisms for ethanol withdrawal-induced anxiety are poorly understood but can be studied using rodent tests of anxiety-related behaviors. While most studies have investigated the effects of chronic exposure, acute withdrawal also induced anxiety-like behaviors in mice after a single administration of ethanol (4.0 g/kg, ip) in the elevated plus maze. The goal of the present experiments was to characterize the effects of acute or repeated administrations of ethanol in mice bidirectionally selectively bred for ethanol withdrawal handling-induced convulsions. In one experiment, WSP-1 and WSR-1 mice were administered a single dose of ethanol (4.0 g/kg) and tested in the elevated plus maze at 16 hrs after injection. No effect of treatment or line was found in anxiety-like behaviors measured by open arm exploration. However, ethanol withdrawal significantly decreased locomotor activity as measured by number of closed arm entries, further evidence that the relationship between anxiety-like behaviors and general activity is complex. In another experiment with WSP-1 and WSR-1 mice, three daily administrations of ethanol (4.0 g/kg) resulted in a trend toward decreased percent open arm entries and a significant effect of line (WSR>WSP) without altering locomotor activity at 16 hrs after the final injection. Other studies compared the selected lines during subchronic withdrawal, and using other assays of anxiety-like behavior. These preliminary results tentatively suggest increased anxiety-like behavior in EtOH-treated animals with a larger effect in the WSR than WSP. Additional studies are necessary to determine the selective effects of ethanol withdrawal on anxiety-like behaviors in the WSP and WSR lines.

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23. Dissecting short- and long-term social and non-social discrimination capacity of mice using chromosome substitution strains

E Pjetri, C Fernandes, H Bruining, H Oppelaar, FJ Meye, GMJ Ramakers, MJH Kas

To identify genetic loci responsible for social recognition and memory, a panel of recently generated chromosome substitution (CS) strains was screened in a social discrimination paradigm. In each of the CS-strains, a single chromosome from the donor strain (A/J) has been substituted in the genetic background of the host strain (C57BL/6J), providing a powerful approach to identify QTLs affecting complex traits (Singer et al., 2004). In the social discrimination paradigm, individual CS-mice were sequentially exposed to one and two intruder mice (the familiar and an unknown intruder) with two inter-exposure intervals (IEI) of 5 minutes and 24 hours. In contrast to C57BL/6J controls, we found CS-strains that did not discriminate between the familiar and unknown intruders after the short- (5 min) or long-term (24 hours) IEI. This suggests that different A/J chromosomes contribute to short- and long-term social discrimination capacity. Furthermore, electrophysiological data revealed that the CS-line with impaired long-term (24 hrs) discrimination capacity has also deficits in the induction of hippocampal long-term potentiation, a hallmark for memory (Kogan et al., 2000; Pastalkova et al., 2006; Whitlock et al., 2006). C57BL/6J and selected CS-strains with either short- or long-term discrimination deficits in a social recognition paradigm were run through a battery of cognitive tasks (social recognition, novel object recognition and the Morris water maze) to assess cognitive performance also in a non-social contexts. Results of this battery will be presented.

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24. Audiogenic epilepsy in mouse and rat after neonatal treatments

NV Markina, OV Perepelkina, TV Timoshenko, AV Revischin, Il Poletaeva

Injections of various drugs in neonatal mouse and rat pups (and pain stimulation as the control treatment as well) induce various differences in the behavior of adult subjects. The degree of these effects and even the presence of absence of them depend on genotype. Audiogenic epilepsy is the physiological trait which varies largely among rat and mouse strains and correlates with the general seizure susceptibility. The pattern of genetic determination varies also not only across species but between mouse strains as well. Neonatal caffeine and piracetam did not affect the penetrance of the audiogenic seizures in DBA/2J mice at the age of 32-35 days. At the same time the duration of seizure fit was smaller and fit severity larger. This means that the trait expressivity changed significantly.

At the same time the pattern of audiogenic fit changes in adult mice of 101/HY strain was different from that of DBA/2J. The most striking result of neonatal Semax injections (synthetic analogue of ACTH4-10 fragment) in CBA mice was the appearance of audiogenic seizures in the adult animals which were never observed in the intact CBAs. The general level of audiogenic susceptibility in rats of KM strain highly susceptible to audiogenic epilepsy did not change as the result of neonatal caffeine treatments although the pattern of the seizure fit t(he involvement of different muscle groups) changed, resulting in the unusual seizure type. Brain neurogenesis (the number of post-mitotic cells) in the area of fascia dentate as the result of neonatal Semax depend on genotype.

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Funding Support: Russian Foundation of Basic Research.

25. Impairments in social interactions, social communication and memory in μ-opioid receptor KO mice: implications for an animal model of autistic spectrum disorders

S Pondiki, MG Di Certo, A Moles, FR D'Amato

Modelling the symptoms of autism and autistic spectrum disorders (ASD) in mice represents a unique challenge. In an effort to elucidate the role of the μ-opioid receptor system in the development of autistic-like behavior in mice, we subjected animals of all three genotypes to a set of tests at different developmental stages.

μ-KO mice displayed a deficit in social interactions as revealed in the social approach/avoidance test. Furthermore KO mice exhibited impaired social communication when tested for ultrasonic vocalizations during separation from the mother under different conditions, a finding that was also sensitive to the genotype of the mother. Ritualistic repetitive behaviors and resistance to change, as measured with the test of novel object recognition and exploration, were also prominent in the KO mice. Regarding other associated symptoms of the ASD, KO mice displayed impaired aversive memory in the active avoidance test and a hyper-responsive HPA axis to psychosocial stressors.

These results, together with previous findings from our laboratory revealing a deficit in attachment behavior in KO mice, render the μ-opioid receptor KO mice a good canditate animal model for the study of autistic and autistic-like behaviors.

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Critical role of actin filament dynamics in the consolidation and re-consolidation of fear memory

K Rehberg, O Stork

Dynamic alterations of actin filaments are critically involved in the morphological reorganisation of neuronal circuits, and are thought to underlie information storage in the brain during memory formation. Several molecular factors have been identified to control such actin filament dynamics in the amygdala during fear memory formation; these include small GTPases and Rho-associated kinase, as well as the serine / threonine kinase Ndr2 (e.g., Lamprecht et al 2002, Stork et al 2004). In the current study we intended to define critical time windows of actin filament dynamics in fear memory formation. To this end, the death cap mushroom (*Amanita phalloides*) protein phalloidin, which prevents actin filament depolymerisation through its binding to F-actin, was injected to the basolateral complex of the mouse amygdala at different time points (30min, 2h, 6h and 24h) after auditory cued fear conditioning. All injections within the first 6h post training resulted in a disturbance of fear memory consolidation, as indicated by pronounced reduction of conditioned freezing behaviour one week after training, whereas phalloidin treatment was without effect when applied at the 24h time point. Injections after retrieval of conditioned fear moreover prevented the re-consolidation of the fear memory. Together these findings suggest the existence of critical time windows of actin filament dynamics in the basolateral amygdala underlying the consolidation and re-consolidation of conditioned fear.

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Gene-environment interaction: behaviour and gene expression responses to environmental stress and antidepressants in four mouse strains

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As part of the European Sixth Framework Programme Integrated Project 'Genome-Based Therapeutic Drugs For Depression (Gendep)', we have compared the behavioural responses of males and females of four inbred mouse strains (C57BL/6J, DBA/2J, 129SvemJ, and FVB/NH) to environmental stress (24 hours maternal separation at 9 days or 14 days of chronic mild stress) and to the drugs escitalopram and nortriptyline (single dose or 14 day course), using the hole board and Porsolt forced swim tests. The five factors (strain (4 levels), sex (2), environment (3), drug (3), dose regime (2)) combine to produce 144 experimental cells, n=9 per cell. This provides a unique opportunity to look for interactions such as strain-specific environmental effects. One such is a C57BL/6- specific, long lasting sensitivity to the effect of maternal separation, visible in the Porsolt test 10 weeks later. We are hybridising hippocampal RNA to Affymetrix microarrays (one animal per cell in the first pass) and will thus be able to examine gene expression correlates of behaviour across strains and environments.

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Mice lacking the microtuble protein STOP show motor coordination deficits

H Schellinck, E Seary, N Butcher, M Wong, A Andrieux

Mice lacking the microtubule protein STOP display hyperdopaminergic and hypoglutamatergic activity as well as behavioural abnormalities in pre-pulse inhibition and social interaction. These characteristics are similar to those observed in humans with schizophrenia. To determine if these mice demonstrate the attention and memory impairments associated with schizophrenia, we assessed their ability to perform in the attention set shifting task and the Morris water maze. The STOP knockouts, heterozygous and wildtype mice performed equally well on the attention set shifting task. In contrast, the STOP mice had difficulty swimming any distance in the Morris water maze and so could not be tested in this paradigm. To determine if motor deficits were responsible for the latter impairment, we investigated their performance on the rotorod. Both male and female STOPs completed fewer rotations than heterozygote and wildtype mice and did not improve across days or trials. Analysis of covariance with weight as the covariate indicated that low weight could be a major factor in this impairment. We are continuing to investigate the motor deficits in STOP knockouts to determine how best to separate motor deficits from other behavioural impairments.

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Funding Support: Natural Sciences and Engineering Research Council of Canada.

Basal and exploratory activity of mice: individual testing versus automated monitoring in a social home cage context

A Sieber[1], F Neuhäusser-Wespy[2,3], P Zinn[2], H-P Lipp[2,], DP Wolfer [1,2]

Basal and exploratory activity of mice are traditionally assessed in test batteries in which animals are tested individually. This approach is labor intensive and inefficient given the large number of genetically modified mouse lines that need to be phenotyped. In addition it yields unreliable results due to the lack of standardization and the stress induced by social isolation of the animals, frequent handling, and exposure to changing testing environments. TraffiCage and the operant learning environment IntelliCage are newly developed fully automated and standardized testing devices that permit to investigate transponder tagged mice directly in their home cage and in a social context. In order to evaluate the suitability of TraffiCage and IntelliCage to assess basal and exploratory activity of mice, we have tested three mouse strains (C57BL6, DBA/2, B6129F1) and a mutant line underexpressing the subunit NR1 of the NMDA receptor first in TraffiCage and IntelliCage and then individually in an established test battery (open field, light/dark box, O-maze, emergence test, object exploration). In order to compare the reproducibility of results, a genetically heterogeneous population (C57BL/6 x 129Sv F2) was tested twice with an interval of 4 months in IntelliCage and in the open field test. Both TraffiCage and IntelliCage readily discriminated the three mouse strains as well as mutants and controls of the NR1 line based on measures of spontaneous activity. Group differences correlated well between TraffiCage and IntelliCage, but were only partial predictors of results obtained in subsequent individual tests, indicating that new protocols in the home cage environment of TraffiCage and IntelliCage need separate validation. Repeated analysis of the F2 population revealed much improved reproducibility of activity measurements obtained in IntelliCage compared to individual testing in the open field.

[1]ETH Zurich, Department of Biology, [2]University of Zurich, Institute of Anatomy, and
[3]NewBehavior AG, Zürich, SWITZERLAND
Funding: NCCR Neural Plasticity and Repair, Swiss NF, European Commission.

Postnatal manipulation reduces anxiety in 5HT1A KO mice

C Zanettini [1], L Lo Iacono[2], V Carola[2], A Moles[1], C Gross[2], FR D'Amato[1]

Gene–environment interactions occur when the effect of exposure to an environmental feature is conditional on the genotype of the individual. Mice lacking the 5-HT1A receptor have been classified as more anxious in comparison with their wildtype controls. To evaluate susceptibility of this genotype to early environmental factors, we exposed these animals to postnatal manipulation. This experimental protocol is known to affect the hypothalamus-pituitary- adrenal axis functioning, and reduce anxiety. Offspring of heterozygous pairs lacking the 5-HT1A receptors were separated from the mother and exposed daily to 15 min of clean bedding, during the first two weeks of life. Adult offspring were tested in the open field and plus maze apparatuses. These animals showed a reduction in anxiety in these tests, with the three genotypes showing a similar trend. Adult females were tested for social anxiety in a resident/intruder paradigm: postnatal manipulation increased resident's ultrasonic calls, suggesting a general increase in social interest. Adult males were tested in the approach/avoidance test: only postnatally manipulated knockout mice showed a significant enhancement of their sociability in comparison to their controls. We suggest that anxiety linked to
social stimuli, rather than exploration of new environment, is more vulnerable to early life events.

[1]CNR Institute of Neuroscience, Roma, and [2]EMBL, Monterotondo (RM), ITALY

32. Towards delineating violence from 'normal' aggressive behavior in mice – a theoretical approach

D Natarajan[1], H de Vries[2], JM Koolhaas[1]

Violence, given its prevalent anthropomorphic identity, is mostly synonymous with escalated, pathological, maladaptive, abnormal, deviant forms of aggression in rodents. Violence research in rodents has been a formidable challenge owing to its species-specific behavioral and physiological constraints and its consonance with human studies. Additionally, excesses of aggression have been suspected far from an objective study, given that mice are despotic societies where dominance is established exclusively by a permissible excessive 'functional' aggressive behavior (dispersive). A logical yet a most profound follow-up have been the identification of this loss of functional/ normal aggression, thus lending a fair degree of ambiguity in this regard. Henceforth a compelling need has arisen to identify the underlying subtle complexities in behavior concerning violence and its delineation from normal aggression in mice. The present study identifies *content* and *context* as the indisputable elements that can be used to collectively describe and distinguish violence from normal to hyper aggression. *Content* includes magnitude of offence (duration; frequency), arousal, ritualistic adherence and sensitivity to the inhibitor submission cues. *Context* includes the discretion in identifying an opponent by nature of its state (free moving/ anaesthetized); environment (home/ neutral territory). Thus the main focus of the study was to identify the *non-inhibitory* and the *in-discriminatory* components in addition to *offence* as key features to look for in violence beyond the realm of *normal* aggression. *Methods :* The availability of high aggressive genetically selected mice models namely SAL, TA, NC900 and the intermediates aLAL, aTNA has made possible the above requisite of investigating violence in rodents objectively. Agonistic encounters between these high aggressive mice and an opponent was taped for 5 mins in the resident home cages for three successive days followed by a neutral cage encounter and analyzed using Observer version 5.0 software. Event log data and transition matrices representative of each mice line, obtained from the above program, have been subjected to extensive theoretical analysis. *Results :* The SAL is invariably a model of violence while other lines are models of *hyper* or *normal* aggression. SAL not only rated the highest in offence but also showed uncontrollable arousal and poor social behavior. It lacked both *inhibition* as well as *discrimination.* The identification of a violent model genetically selected from a wild type house mouse population has now opened up possibilities of behavioral specificities of anti-aggressive drugs at a preclinical level in rodents' representative of *normal* and *docile* strains.

[1]Department of Animal Physiology, University of Groningen, and [2]Behavioural Biology, University of Utrecht, THE NETHERLANDS

7.00 pm: Barbecue dinner

Friday, May 24

8 am: Breakfast

8.45 am: Local Organizing Committee plenary lecture: SDM Brown

introduced by Leo Schalkwyk

The functional annotation of the mouse genome - the challenge of phenotyping

SDM Brown and the EUMORPHIA and EUMODIC Consortia

With the completion of the mouse genome sequence, a key goal for functional genomics is the creation of a series of mutant alleles for every mammalian gene. An even greater challenge will be the determination of phenotypic outcomes for each mutation. A vital element of this endeavour will be to develop standardised phenotyping platforms that allow for reproducibility of test outcome over both time and place. The EUMORPHIA programme, funded by the European Commission, is a consortium of 18 research institutes from across Europe working on establishing new approaches to phenotyping with a focus on improving and standardising phenotyping platforms for the mouse. A major achievement has been the development of a new robust primary screening strategy, EMPReSS (European Mouse Phenotyping Resource for Standardised Screens). This primary screen incorporates over 150 SOPs, many validated on a cohort of inbred strains across a number of laboratories. EMPReSS covers all of the major body systems, as well as generic approaches in imaging, pathology and gene expression. The availability of standardised screens and associated informatics structures and tools will be a vital underpinning for a systematic and rational functional annotation of the mouse genome. Representing phenotypic information in a standardised way presents further challenges. Development of phenotype ontological structures that take into account assay protocol, genetic background and environment will be crucial. In addition, the mining of phenotypic characters for correlations indicative of underlying processes will require the availability of databases of raw phenotype data. In the EUMODIC programme, we will use a version of EMPReSS, EMPReSSslim, to begin the process of primary phenotyping of large numbers of mouse mutants generated through the EUCOMM mouse mutagenesis programme. 4 mouse clinics within the EUMODIC consortium will undertake comprehensive phenotyping using EMPReSSslim of up to 650 mouse mutant lines. A proportion of mutants with interesting phenotypes will undergo more detailed secondary/tertiary phenotyping at other centres within the EUMODIC consortium. All phenotype data will be made publicly available through the EuroPhenome database. EUMODIC is a first step towards tackling the need for comprehensive large-scale phenotyping in the mouse and the study of mammalian gene function.

MRC Mammalian Genetics Unit, Harwell, OX11 ORD, UK

10.15 am – 12.15 pm: Symposium 5

New insight in molecular mechanisms of synaptic plasticity and multiple memory systems

Chairs Melly Oitzl & Harm Krugers

10.15 am: Kamila Markram

Distinct role of the polysialylated neural cell adhesion molecule (PSA-NCAM) in cue and context fear conditioning

K Markram, M Angel L Fernandez, N Abrous, C Sandi

PSA-NCAM, expressed at the synapse, is crucially involved in the formation well as elimination of synapses that are related to memory consolidation processes in several brain structures. The involvement of PSA-NCAM in fear conditioning was investigated by (i) targeted PSA-NCAM cleavage in the amygdala and hippocampus and (ii) general PSA-NCAM cleavage using transgenic mice lacking PSA-NCAM throughout the brain. Both approaches revealed that amygdaloid PSA-NCAM is not required for the formation of cued fear memories. On the contrary, the formation of contextual fear memories requires hippocampal PSA-NCAM. These results suggest distinct mechanisms for fear memory formation in amygdala and hippocampus.

Brain Mind Insitute, Ecole Polytechnique Fédérale, Swiss Federal Institute of Technology, SWITZERLAND
Funding Support: This work was partially supported by grants from the Swiss National Science Foundation (3100A0-108102), the EU 6th Framework Programme (PROMEMORIA LSHM-CT-2005-512012) and INSERM.

10.40 am: Oliver Stork

Somatostatin: a novel molecular mechanisms of fear memory consolidation in amygdala and hippocampus

O Stork, A Albrecht, C Stoppel, C Kluge, H-C Pape

Somatostatin is expressed in a subpopulation of GABAergic interneurons in the amygdala and hippocampus. Although ample evidence suggests that somatostatin plays an important role in a range of hippocampus dependent memory tasks, its contribution to contextual and cued fear memory formation remain to be clarified. Quantification with laser dissection and real time PCR revealed specific changes of somatostatin mRNA expression in subregions of the amygdala and hippocampus following fear conditioning. Moreover, genetic ablation and acute pharmacological depletion of somatostatin both impaired contextual fear conditioning and hippocampal long-term potentiation. Together these results indicate a critical role of somatostatin for information storage in the amygdalo-hippocampal system.

Otto-von-Guericke University Magdeburg, GERMANY, and Institute of Psychiatry, King's College London, UK
Funding Support: German Research Foundation.

11.05 am: Harm Krugers

Hormonal control of molecular mechanisms involved in learning and memory

HJ Krugers, L Conboy, O Wiegert, D Holman, JM Henley, C Sandi, M Joëls, CC Hoogenraad

Emotionally arousing and stressful events are remembered well in general. These memories are facilitated by corticosteroid hormones, which are released during the stressful situation. One of the questions is how these hormones exert their effects. AMPA receptors are critically involved in synaptic efficacy, plasticity and learning and memory processes. Electrophysiological data indicates that corticosteroid hormones, via activation of the glucocorticoid receptor increases AMPA receptor mediated synaptic transmission in ventral tegmental area and hippocampus. Accordingly, we will present evidence that these hormones induce trafficking of AMPA receptors to the membrane. These effects mediated via glucocorticoid receptor and involve a genomic action.

SILS-CNS, University of Amsterdam, THE NETHERLANDS

11.30 am: Thomas Steckler

Modulation of glutamatergic mechanisms in learning and memory - focus on metabotropic glutamate receptors

T Steckler

The metabotropic glutamate receptor (mGluR) family consist of a group of eight G-protein-coupled receptors that differ in their function, distribution and roles within the brain. Various mGluR subtypes have been suggested as targets for the development of novel, promising pharmacological treatment opportunities for a wide range of psychiatric disorders, including anxiety, depression and schizophrenia. Modulation of the glutamatergic system via activation or inhibition of different mGluR subtypes has also been shown to affect cognitive function. Cognitive side effects are limiting in the development of novel anxiolytic, antidepressive or antipsychotic drugs. Therefore, a better understanding of the cognitive effects of manipulations of the mGluRs on learning and memory is mandatory. The role of the various mGluRs in the modulation of cognitive function will be discussed, with a focus on mGluR1, mGluR2 and mGluR5 receptors, using data from both pharmacological and knockout studies. Furthermore, the advantages of allosteric modulation of the mGluRs (in contrast to orthosteric modulation) will be discussed.

Department of Psychiatry, R&D Europe, Johnson & Johnson PRD, Beerse, BELGIUM

11.55 am: General discussion

12:30pm- 7:00 pm: Social Program

visits to Burgers Zoo, Museum Kröller-Müller (or Arnhem station for those wishing to go further afield). Packed lunch supplied.

7.00 pm: Dinner

Friday, May 25

8 am: Breakfast

8.45 – 10.45 am: Symposium 6

Neurobehavioral genetics of marijuana use and schizophrenia

Chairs Emmanuel Onaivi & Hiroki Ishiguro,

8.45 am: Ester Fride

Early exposure to stress and/or marijuana: Implications for the appearance of schizophrenia later in life

E Fride

Marijuana smoking during adolescence has been associated with increased vulnerability to develop schizophrenia, yet in many cases no psychopathological symptoms erupt. In our work we investigated prenatal roots of schizophrenia, as a function of exposure to delta-9-tetrahydrocannabinol (THC), the major psychoactive ingredient of marijuana (Cannabis sativa) or to maternal stress. Children of marijuana smoking mothers have been shown to display mild impairment of executive functioning known to be regulated by the prefrontal cortex. Dopaminergic overactivity in the prefrontal cortex is an important hallmark of schizophrenic behavior. Interestingly, one of the important parallels between prenatal stress exposure and prenatal exposure to THC includes selective enhancement of activity of dopaminergic neurons in the mesocortical pathway (the neural pathway leading to the prefrontal cortex). Moreover, the 'endocannabinoid CB receptor system' (ie., the cannabinoid receptors and their endogenous ligands such as anandamide and 2-arachidonoyl glycerol) have now been shown to be intricately involved in the acute and chronic stress response. We investigated therefore whether (in mice) prenatal exposure to stress or to THC will induce alterations in the endocannabinoid CB receptor system during and/or after maturation, thereby influencing the risk for developing schizophrenia. Our findings indicate that prenatal exposure to THC induced an enhanced 'cannabinoid tone' (behavioral and CB1 receptor changes) while prenatal stress induced alterations in the vulnerability to cannabinoids with respect to the regulation of prepulse inhibition, a diagnostic measure of schizophrenia. In conclusion, we suggest that the endocannabinoids and their receptors are important mediators in translating developmental vulnerabilities into the expression of schizophrenia.

Department of Behavioral Sciences, College of Judea and Samaria, Ariel, ISRAEL
Funding Support: National Institute of Psychobiology in Israel.

9.10 am: Hiroki Ishiguro

Genetic profiling in schizophrenia.

H Ishiguro, T Arinami

Clinical studies have implicated that cannabis consumption could be a self-medication therapy for counterbalancing negative symptoms of schizophrenia or side-effects of antipsychotic treatment. Contrary to this, epidemiologic research suggest that cannabis use, including maternal use, is a risk factors for developing psychosis and that the use of marijuana may worsen psychotic symptoms of schizophrenia. Schizophrenia is a polygenic disease with influences from genetic and environmental factors. Therefore, it is hypothesized that genetic components related to endocannabinoid system in brain and/or genes whose expression/function are regulated by environments, such as cannabis use, may underlie schizophrenia. Thus gene profiling based on these two hypotheses may give us new knowledge in understanding schizophrenia. Variations in the Cannabinoid CB1 receptor (CNR1) gene have been reported to be associated with schizophrenia. Furthermore CB1 antagonist was shown to counteract a phencyclidine-induced deficit of PPI in mice. We hypothesized that other components of endocannabinoid system might also have roles in schizophrenia. Cannabis also activates cannabinoid CB2 receptor (CNR2) gene, and we have recently demonstrated that CB2 receptors are expressed in brain and may be involved in stress-related behavior. Several cannabinoid receptor ligands have been reported to bind to the putative GPR55 cannabinoid receptor. We do not have an evidence for associations between polymorphisms in CNR2 or GPR55 and schizophrenia. Cannabis consumption during pregnancy has been reported to alter neural adhesion molecule (CAM) L1 in fetal brain and previous studies indicated associations between L1 and schizophrenia, we have focused our studies on NrCAM which is one of CAM super family that is closely related with L1 CAM. Thus, an association study between NrCAM and schizophrenia and behavioral tests in Nrcam knockout mice, could provide new knowledge in schizophrenia.

Department of Medical Genetics, University of Tsukuba, JAPAN
Funding Support: Japan Science and Technology & Scientific Research on Priority Areas, Research on Pathomechanisms of Brain disorders from the Ministry of Education, Culture, Sports, Science and Technology of Japan (18023009 and 18790823).

9.35 am: Makoto Takahashi

Brain-Derived Neurotrophic Factors (BDNF) and cytokines in schizophrenia.

M Takahashi

Neurotrophins and cytokines mediate gene-environment interactions and are implicated in neuropsychiatric disorders. BDNF is regulated via glutamate receptors in an activity-dependent manner and plays a role in synaptic development and plasticity. To elucidate how BDNF is involved in neuropsychiatric pathophysiology, we examined an impact of NMDA receptor blockade on neurobehavioral performance and BDNF levels in the brain. Early postnatal exposure to PCP caused several behavioral changes during adulthood; hyperlocomotion, poor learning in active avoidance, decrease in latent inhibition of contextual fear conditioning, and decrease in social interaction. These behavioral changes were ameliorated by atypical antipsychotic drug clozapine. On the other hand, postnatal PCP treatment induced long-lasting increase of BDNF levels in the hippocampus. The increase was accompanied with an increase in NR1 glutamate receptor subunit and a decrease in TrkB receptor levels. Perturbed synaptic plasticity was suggested since PCP decreased phosphorylation of MAPK and CaMKII. Clozapine reversed PCP-induced decrease of TrkB receptor levels and normalized phosphorylation levels of MAPK, although CaMKII hypophosphorylation was not affected by clozapine. Decreased levels of TrkB and upregulation of BDNF has been reported in postmortem brains of patients with chronic schizophrenia. Animal studies of postnatal PCP exposure suggest that environmental risk factors which influence glutamatergic system contribute to the pathophysiology of schizophrenia probably through abnormal BDNF/TrkB signaling.

Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, Niigata, JAPAN

10.00 am: Emmanuel Onaivi

Endocannabinoid receptor genetics and marijuana use

ES Onaivi

No drug has conjured myths and controversy as marijuana. Schizophrenia is an elusive brain disorder because of its complexity and polygenic nature. A number of candidate genes have been associated with schizophrenia but no single gene has been replicated. The link between marijuana use and schizophrenia has also been controversial. However, many studies have shown that the use of marijuana may induce psychosis in vulnerable individuals and worsen psychotic symptoms in schizophrenic patients. New knowledge has started to shed some light on the molecular mechanisms of the effects of marijuana use and schizophrenia. Such new research indicates that one of the most abundant receptor binding sites in the brain is for marijuana active ingredients called cannabinoids. The human body and brain makes its own marijuana-like substances that are called endocannabinoids that binds to cannabinoid receptors. The discovery of endocannabinoid physiological control system has led to the examination of this system in CNS and its role in mental disorders including schizophrenia and drug dependency. Thus, cannabinoids, endocannabinoids and marijuana use activate two well characterized receptors encoded by CNR1 and CNR2 cannabinoid receptor genes. It is therefore possible to test the association between marijuana use and schizophrenia by studying the evidence for the cannabinoid hypothesis of schizophrenia. However, CB2 cannabinoid receptors were thought to be mainly expressed in the periphery and in immune cells. Our data indicate neuronal CB2 receptors in the brain. Further evidence is presented for the involvement of neuronal CB2 cannabinoid receptors in neuropsychiatric disturbances. The data adds to previous results on role of CNS effects of CB1 receptors and demonstrates the functional expression of CB2 cannabinoid receptors in brain that may provide novel targets for the effects of cannabinoids in mental disorders beyond neuro-immunocannabinoid activity.

Department of Biology, William Paterson University, Wayne, New Jersey, USA
Funding Support: William Paterson Center for Research.

10.25 am: General discussion

10.45 am: Coffee

11.15 am: Young Investigator Awardee lecture:Elissa Chesler

introduced by Robert Williams:

Ontological Discovery from Genes to Behavior

E Chesler

A fundamental research challenge for behavioral neuroscience is to interpret relationships among behaviors, and to identify behaviors that share a biological substrate. Because the attributes we study are typically defined using phenomenology, they do not always map onto the underlying biological substrates, nor do behavioral classification systems necessarily map onto essential drivers of behavioral categories. Systems biological approaches, including the development of high-throughput molecular profiling, have lead to a tremendous wealth of data that can be exploited to understand the ontology of behavior. Genetic approaches, including genetic correlations of behavior in genetic reference populations, have been successfully deployed web based tools such as GeneNetwork.org. Gene-phenotype associations can also be ascribed using genetic correlation. These associations can be driven purely by genetic architecture, and must be validated using orthogonal populations or alternative experimental methods, including differential expression analysis, literature mining and mutant phenotyping. By mining gene-phenotype associations across behaviors and experiments, we can define behaviors that share a similar biological basis. In this way, complex behaviors can be placed in the context of other neurobiological phenotypes, and behavioral assays can be compared across model organisms. this approach is implemented as a web-based tool called "Ontological Discovery Environment."

Oak Ridge National Laboratory, Oak Ridge, Tennessee, and Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, Tennessee, USA

12.15 pm: Lunch

1.45 – 3.45 pm: Symposium 7

Genetic Approaches to Study Depression and the Antidepressant response,

Chairs John Cryan, University College Cork, Ireland & Stephanie Dulawa, University of Chicago, USA

1.45 pm: John Cryan

In search of depressed mouse: Using genetically modified mice to understand depression

JF Cryan

Psychiatry has proven to be among the least penetrable clinical disciplines for the development of satisfactory in vivo model systems for evaluating novel treatment approaches. However, mood disorders remain poorly understood and inadequately treated. The ability to genetically modify mice has been one of the major breakthroughs in modern medical science affecting every discipline including psychiatry. It is hoped that the application of such technologies will result in the identification of novel targets for the treatment of diseases such as depression and to gain a better understanding of the molecular pathophysiological mechanisms that are regulated by current clinically effective antidepressant medications. The advent of these tools has resulted in the need to adopt, refine and develop mouse specific models for analyses of depression-like behaviour or behavioural patterns modulated by antidepressants. Moreover, the full potential of regionally selective and inducible knockout and transgenic mice and siRNA technology has yet to be realized, but such strategies offer many advantages over currently used techniques. Such mice certainly will be welcome tools to dissect regionally specific circuits that might influence the actions of antidepressants. Using specific examples of mice with targeted deletions of glutamate, GABA or noradrenergic system, the utility of current models and research strategies aimed at investigating novel targets relevant to depression in the mouse will be discussed. Key questions that are considered relevant for examining the utility of such models will be addressed. Further, we describe other avenues of research that may give clues as to whether indeed a genetically modified animal has alterations relevant to clinical depression. Overall, it is prudent and most appropriate to use convergent tests that draw on different antidepressant-related endophenotypes, and complimentary physiological analyses in order to provide a program of information concerning whether a given phenotype is functionally relevant to depression-related pathology.

School of Pharmacy, University College Cork, Cork, IRELAND

2.10 pm: Stephanie Dulawa

Genetic mechanisms underlying the behavioral response to chronic antidepressant treatment: insights from new mouse models

SC Dulawa, AM Nitzke

The onset of the therapeutic response to antidepressant treatment exhibits a characteristic delay. Animal models in which behavioral responses to antidepressants emerge following chronic, but not subchronic, treatment have remained elusive. Such models are required to study the mechanisms underlying the therapeutic effects of antidepressant treatment. We have investigated the behavioral effects of chronic treatment with selective serotonin reuptake inhibitors (SSRIs) in a broad panel of inbred mouse strains. Mice were evaluated for anxiety- and depression-related behavior in the open field, forced swim, and tail suspension tests following three weeks of SSRI administration in the drinking water. We found that only certain inbred strains, including the Balb/cJ, showed reduced anxiety- and depression-related behavior in response to chronic, but not subchronic, SSRI treatment. Our findings show that certain inbred mouse strains can be utilized to study the therapeutic effects of chronic antidepressant treatment, without any stress-inducing procedures. Studies investigating the role of specific serotonin receptors in the response to chronic SSRI treatment are currently underway.

Department of Psychiatry, University of Chicago, Chicago, Illinois, USA

2.35 pm: Inga Neumann

Selection for high versus low anxiety: rat model for anxiety, depression and high aggression

ID Neumann

The selective breeding of Wistar rats for high (HAB) versus low (LAB) anxiety-related behaviour since more than 12 years resulted in two rat strains which are well established and characterized as a suitable animal model of anxiety- and depression-related disorders. The robust differences in the anxiety phenotype are accompanied by alterations in neuroendocrine and neuronal stress-responsiveness to various stimuli, and in relevant brain neurotransmitter systems including arginine vasopressin (AVP), CRF and serotonin. Importantly, a SNP has been identified in the AVP gene promoter of the HAB rats resulting in up-regulation of vasopressin gene expression specifically within the hypothalamic PVN. Accordingly, local blockade of AVP synthesis and actions reverses the high level of anxiety in HAB rats. HAB and LAB rats also provide an excellent model for studying interactions between early environmental factors (i.e. early life stress: prenatal stress, maternal separation) and the genetic predisposition for either high or low stress susceptibility. Thus, opposite effects of prenatal or postnatal stress on adult emotionality, stress coping and neuropeptide expression patterns within the hypothalamus have been found in adult HAB and LAB rats. Finally, selection for low trait anxiety in LAB rats goes along with the development of high intermale aggression during the resident-intruder test, and a high neuroendocrine and neuronal response to social stimuli. Therefore, LAB males may develop as a promisising animal model for studying neurobiological mechanisms of pathological aggression.

Department of Behavioural and Molecular Neuroendocrinology, University of Regensburg, Regensburg, GERMANY
Supported by VolkswagenStiftung.

3.00 pm: John Quinn

Molecular genetics of human monoaminergic transporters: relevance to behavioral disorders and drug response

JP Quinn, K Haddley, S Vasiliou, FR Ali, VJ Bubb

Polymorphisms found in non-coding sequences are implicated in the aetiology of mental illness. We have demonstrated that some members of a subclass of such polymorphisms termed Variable Number Tandem Repeats (VNTRs) have functional effects on gene expression. This suggests that individuals with a particular combination of polymorphisms may respond differently to an individual medication or environmental stress. We have demonstrated in both the human serotonin transporter gene (5HTT) and the dopamine transporter gene (DAT1) that specific VNTRs, correlated with predisposition to neurological and psychiatric disorders, act as transcriptional regulatory domains. These domains can act as both tissue-specific and stimulus-inducible regulators of gene expression. Further, in vitro, the 5HTT VNTR in intron 2 is responsive to lithium and the DAT1 VNTR in intron 8 modulates a response to cocaine and amphetamine. These functional studies link the clinical findings that at risk groups have a higher incidence of a particular variant of polymorphism. In the above examples, the VNTRs may alter the concentration of transporter protein in specific cells or in response to a challenge; chemical, environmental or physiological. We have demonstrated that multiple VNTRs within the same gene or distinct genes can bind the same transcription factor and therefore potentially are on the same signal transduction pathway. Therefore a more global analysis of VNTRs complemented by single nucleotide polymorphism (SNP) data correlated with disease predisposition would be a first step to understand the integrated cellular response to a specific challenge. Further, it is predicted that this data will be invaluable to enable future intelligent directed drug design.

School of Biomedical Science, University of Liverpool, Liverpool, UK
Funded by the BBSRC and Wellcome Trust.

3.25 pm: General discussion

3.45 pm: Break

4.00 pm: Selected papers session:

Chair Andrew Holmes

4.00 pm: Lisa Tarantino

Identification of an ENU-induced mutant that displays hyperactivity in a novel environment, exaggerated responses to psychostimulants and a prolonged stress response

JS Bailey, L Grabowski-Boase, BM Steffy, T Wiltshire, LM Tarantino

We have identified an ENU mutant, *Highper*, that displays altered locomotor response in the open field that is greater than 3.5 standard deviations above the mean activity levels of wildtype mice. The *Highper* mutation is recessive with a penetrance of approximately 50%. The *Highper mutant also* shows an exaggerated locomotor response to psychostimulants. Locomotor activation in response to 20mg/kg cocaine is two-fold higher in *Highper* mutants and locomotor response to 30mg/kg methylphenidate is fifty percent higher in *Highper* mutants. *Highper* mutants also have a prolonged elevation of corticosterone levels in the short-term restraint model of acute stress response. However, preliminary studies indicate that these elevated levels of corticosterone in *HIghper* mice may not explain the differences in locomotor response to cocaine. Finally, *Highper* mice appear to be more sensitive to the rewarding effects of cocaine. In the conditioned place preference assay, *Highper* mice spend more time in the cocaine-paired chamber than do wildtype mice. Cocaine self-administration studies are planned to further examine the reinforcing effects of cocaine in *Highper* mice.

The identification of ENU induced mutations in behavioral screens has been hampered by the phenotypic variability introduced by outcrossing to mapping strains. We have developed SNP marker panels for inbred strains that are closely related to B6 to reduce the number of background loci that may interfere with our phenotype. We have also developed a unique breeding strategy to limit the genetic contribution of the mapping strain using a mapping population generated by backcrossing F1 progeny to an affected B6 animal. Affected animals are clearly identified in this N2 population when compared to a control N2 cross. SNP genotyping has successfully identified an associated genomic locus with relatively few mapping cross animals, and analysis of additional mapping cross animals is currently underway to confirm and further define the map location.

Genomics Institute of the Novartis Research Foundation, San Diego, California, USA

4.15 pm: Igor Ponomarev

Alcohol-induced changes in global gene expression in a mouse model of binge drinking

I Ponomarev[1], JS Rhodes[2], JC Crabbe,[3] RA Harris[1]

Excessive alcohol consumption is a prerequisite for the development of alcohol dependence. Therefore, it is important to identify molecular targets affected by high alcohol intake in order to understand the mechanisms of alcoholism progression, which mediate the switch from controlled alcohol consumption to alcohol abuse and alcohol dependence. Our main goal was to examine the effects of voluntary alcohol consumption on brain gene expression profiles using a mouse model of binge drinking. We used a modification of a “Drinking In the Dark” (DID) procedure (Rhodes et al., 2005) that allows mice to experience physiologically relevant amounts of alcohol in a non-stressful environment and also allows for detection of alcohol-sensitive genes in a dose-dependent manner. C57BL/6J male mice were exposed to either 20% ethanol solution or water (single bottle) starting 3 hr after lights off for 4 hrs and brains were harvested immediately after the drinking session. cDNA microarrays were used to assess the effects of four variables (consumption during the first 2 hrs, consumption during the last 2 hrs, total consumption and blood alcohol concentration at the end of drinking) on global gene expression in six brain regions. As an initial step we characterized alcohol-related gene expression profiles in cerebellum, a structure with well defined neurocircuitry, which is involved in many alcohol-related behaviors. Results showed that 1) consumption during the first 2 hrs was the best predictor of alcohol-induced changes in global gene expression, 2) most transcripts significantly correlated with alcohol drinking showed negative correlations (~85%), indicating general transcriptional suppression in response to alcohol, and 3) many alcohol-sensitive genes (*e.g. Fabp7, Sox2, Sox7* and gephyrin) were previously shown to be expressed in a cell type – specific fashion suggesting that different cells may use different strategies to adapt to alcohol exposure. The long term goal of this research is to determine molecular mechanisms of cellular adaptation to alcohol in individual neuronal populations of the neurocircuits that mediate alcohol actions.

[1]Waggoner Center for Alcohol and Addiction Research, University of Texas at Austin, Austin, TX, USA; [2]University of Illinois at Urbana-Champaign, Champaign, IL, USA; [3]Portland Alcohol Research Center, Oregon Health & Science University and VA Medical Center, Portland, OR, USA
Supported by NIAAA grants from the Integrated Neuroscience Initiative on Alcoholism (AA UO1 13520, AA UO1 13518, AAUO1 13475; INIA Pilot Projects) and the Department of Veterans Affairs.

4.30 pm: Derrick Nehrenberg

Mice selectively bred for high aggression reproduce key features of emotional dysregulation

DL Nehrenberg[1,2], RM Rodriguez[4], S Moy[3], JM Lauder[1,2], WC Wetsel[4]

Because human impulsive aggression has been attributed to emotional dysregulation, we examined whether mice selectively bred for high aggression (NC900) reproduced behavioral symptoms and neurobiological features of emotional disorders compared to mice bred for low-aggression (NC100). First, we used the social preference test to determine whether NC900 aggression is akin to human impulsive, offensive aggression. When given a choice, NC900 prefer to engage enclosed social partners with high levels of tail rattling and biting behaviors, indicating that their aggression is offensive, rather than defensive in nature. Second, we evaluated whether NC900 would reproduce key features of human anxiety. NC900 animals exhibited more anxiety-like behaviors in a number of tests, were less sensitive to the anxiolytic effects of diazepam, showed less diazepam-sensitive $GABA_A$ binding in multiple brain areas that regulate emotions, and showed less protein levels of the $GABA_A$ $\alpha 2$ subunit, which has been implicated in mediating the anxiolytic effects of diazepam receptor subunit, in the frontal cortex and amygdala. Third, we evaluated whether NC900 mice would exhibit key features of human depression. We found that NC900 animals exhibited more depression-like behaviors in the tail-suspension and forced swim test and exhibited developmental deficits in the number of serotonin cells in raphe nuclei that persisted into adulthood. All together, our results indicate that NC900 aggression is associated with key features of human anxiety and depression, suggesting that their aggression is attributable to alterations in emotion regulation. The presence of aggression, anxiety, and depression phenotypes in one mouse strain is unprecedented. Therefore, we propose that the NC900 animals represent a unique tool to investigate the genetic architecture of emotional dysregulation.

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This work was supported by NIH grants T32 ES007126 and MRDDRC P30 HD031108.

4.45 pm: Aki Takahashi

Forward genetics approach toward complex traits using consomic mouse strains established from C57BL/6J and wild-derived MSM

A Takahashi [1], A Ishii [1,3], A Nishi [1,3], T Shiroishi [2,3], T Koide [1,3]

Individual differences in behavior are arising from quantitative genetic variations in addition to environmental factors. We aimed to reveal those genetic mechanisms underlying individual divergence of behavior by using consomic strains (CSSs) of mouse established from C57BL/6J and MSM. By examining a panel of CSSs on many behavioral traits, such as spontaneous activity, anxiety-like behavior, pain sensitivity, and social behavior, we systematically mapped the chromosomes that have a locus or loci affecting those phenotypes.

To dissect complex trait into fine genetic element, we focused on one strain B6-17MSM, which have substituted chromosome 17 from MSM. B6-17MSM showed reduced novelty-induced activity and increased risk-assessment behavior, but no differences in their home-cage activity or motor coordination compared to C57BL/6J. They also showed increased fear memory in the fear conditioning test. Thus, it was expected that there are genetic locus/loci related to emotionality on the chromosome 17. They also exhibited highly increased social interaction behavior. In addition, we found that B6-17MSM had “hydrocephalus-like” enlarged brain ventricle size. To identify genetic loci related to those phenotypes, we established a series of congenic mouse strains of B6-17MSM. Analysis of congenic strains successfully revealed two novel genetic loci for the brain ventricle size in the proximal region of chromosome 17. In contrast, our result suggested that genetic interaction between two or more loci is required to show increased social contact as B6-17MSM. There are multiple loci for the behaviors associated with novelty-induced activity and two genetic loci for risk-assessment. We focused on one locus around telomeric region, which had as strong effect on the emotionality-related traits as B6-17MSM but independent from hydrocephalus phenotype, and are trying to narrow this locus down to identify the gene.

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5.00 pm: Michael Parsons

Candidate gene association studies for anxiety, activity and cognitive performance in mice

MJ Parsons, L Liu, C Fernandes, JL Paya-Cano, LC Schalkwyk

The genetic components influencing individual differences in behaviour are likely to be complex. We recently conducted association studies for over 170 SNPs both in house and from public databases. The association studies were conducted in an outbred population of 680 Heterogeneous Stock (HS) mice for numerous anxiety, activity and cognition-related measures taken from an extensive behaviour battery including the following behavioural tasks: spontaneous activity in the home cage, open field, elevated plus maze, light/dark box, SHIRPA primary screen, novel object recognition, puzzle box and Morris water maze. Numerous SNPs yielded significant associations with both anxiety and activity measures. Using the WebQTL tool (www.genenetwork.org), we further investigated these genes by correlating their hippocampal mRNA expression levels with these behaviour traits in BXD recombinant inbred mice (n=254, 24 lines). We additionally conducted in silico expression QTL mapping for some of the genes of interest using the WebQTL tool to look for eQTLs that may additionally influence these behavioural measures. We were able to successfully validate these associations, using a converging method, for a number of genes including two glutamatergic genes (Grid1 and Grik4) and a serine/threonine kinase gene (Stk31). Further studies are necessary to elucidate the specific role that these genes play in these phenotypes.

Institute of Psychiatry, King's College London, UK
Funding Support: MRC (UK).

5.15 pm: Annetrude de Mooij-van Malsen

Genetic dissection of avoidance behavior and motor activity levels using chromosome substitution strains of mice

JG de Mooij-van Malsen[1], H van Lith[3], H Oppelaar[1], J Hendriks[1], B Olivier[1,2], MJH Kas[1]

Mood and anxiety disorders are the most prevalent of all psychiatric disorders. Our research project focuses on the genetic dissection of certain behavioural phenotypes, specifically hyperactivity and avoidance behaviour, with the aim to search for more selective pharmacological treatments of neurobehavioral disorders. Therefore, we developed a system for automated multi-day recordings in a designed home cage environment, assessing the animals' reduced preference for exposed areas independent of motor activity levels. Following behavioral testing of chromosome substitution strains of mice in this setup, we were able to map different genetic loci specific for both motor activity levels and avoidance behaviour. Consecutive anatomical research revealed significant changes in the motor cortex and motor neurons of the affected chromosome substitution strain, possibly responsible for the change in motor activity levels. In addition, the genetic locus for mouse avoidance behaviour was syntenic with a human linkage region for emotional disorders. The specific role and associated genetic pathways in central nervous system functioning of candidate genes in these loci are currently being examined.

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5.30 pm: Daniel Nätt

Transmission of stress-induced learning impairment and associated brain gene expression from parents to offspring in chickens

C Lindqvist[1], A Janczak[2], D Nätt[1], I Baranowska[1]*, N Lindqvist[1], A Wichman[3] , J Lundeberg[4], J Lindberg[4], P Torjesen[5], P Jensen[1]

Stress influences many aspects of animal behaviour and is a major factor driving populations to adapt to new niches, such as during domestication. Transmission of stress responses to offspring is known to occur through non-genetic mechanisms, but recent research indicates that inherited epigenetic marking could possibly offer a route for this. Chickens of red junglefowl (RJF; ancestors of modern chickens) and domesticated White Leghorn (WL) were raised in a stressful environment (unpredictable light-dark rhythm), leading to a reduced ability to solve a spatial learning task compared to a non-stressed control group. Offspring of stressed WL, but not RJF, raised without parental contact, had a reduced spatial learning ability and an increased ability to compete for food compared to offspring of the control group. Using a cDNA microarray, we found that in offspring of stressed WL, but not RJF, at least 31 genes were up- or down-regulated in the hypothalamus and pituitary c ompared to offspring of non-stressed parents. Among those, brain-derived neurotrophic factor (BDNF) and nuclear factor kappaB (NFKB) have earlier been associated with spatial learning ability. Additionally, genes that were differentially expressed in the parents tended to be so in the offspring too. Our results may suggest that the gene expression pattern, and the behavioural stress response, was inherited and that the ability to transmit epigenetic information and behaviour modifications between generations may have been favoured by domestication.

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Funding Support: Wallenberg Consortium North, the Swedish Reseach Council for Environment, Agricultural Sciences and Spatial Planning, the Swedish Research Council, the Norwegian Research Council and the Norwegian Centre of Poultry Science.

5.45 pm: Reinald Fundele

Gene expression underlying differential maternal behavior in genetically identical female mice

Gene expression underlying differential maternal behaviour in genetically identical female mice

Y Yu[1], W Shi[1], U Singh[1], KD Broad[2], PB Singh[3], EB Keverne[2], RH Fundele[1]

Interspecies hybridization in mice leads to loss-of-imprinting and increased expression of the imprinted gene *Peg1*, which is important in regulating maternal behaviour as shown by loss-of-function targeted mutation of the gene. Therefore, maternal behaviour in virgin F1 females, derived from matings between the two inbred strain C57BL/6 and SPRET/Ei, was tested to determine whether *Peg1* gain of function alters maternal behaviour. A subset of females exhibited maternal behaviour, whereas other females attacked and killed pups. To identify the genes underlying these differential behaviours in genetically identical females, gene expression was analysed by microarray hybridisation. Four way hybridisations were performed, comparing gene expression patterns in the brains of infanticidal vs. maternal females after [1] and without [2] exposure to alien pups and in both infanticidal and normal females after exposure [3] vs. without [4] exposure to alien pups. These microarray hybridisation experiments yielded a large number of differentially expressed genes. A sub-set of these genes had been shown by gene targeting to be involved in control of maternal behaviour, however no correlation between *Peg1* loss-of-imprinting and maternal/infanticidal behaviour was detected. Interestingly, one gene identified in hybridisation 2 (infanticidal vs. maternal without exposure) is involved in epigenetic control of gene expression. Loss- and gain-of-function mouse models and chromatin immunoprecipitation will be applied to investigate the putative function of this gene in behaviour control.

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This work was supported by grants of the Swedish Research Council and the Wallenberg Consortium North (WCN).

6.00 pm: IBANGS business meeting; open to all members.

7.30 pm: Closing banquet

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