INTERNATIONAL BEHAVIOURAL AND NEURAL GENETICS SOCIETY

FOURTH ANNUAL GENERAL MEETING
November 7-10, 2001
Island Palms Hotel
San Diego, California, USA

Program

Wednesday, November 7

7:00-9:00 PM Registration and reception with cash bar and Hors D'oeuvres

Thursday, November 8 - Grand Marina Room

8:30 AM Welcome and Introduction

8:45 AM *Symposium: DNA Microarrays: A New Tool to Discover Genes Relevant to Learning and Memory*
Chair: J. Kinney, NIMH, Bethesda, Organizers, J.N. Crawley, J. Kinney

8:50 AM M. Mayford, Scripps Research Institute, La Jolla. Microarrays, mutants and memory

9:20 AM J. Dubnau, Cold Spring Harbor Laboratories. Functional genomics of memory in Drosophila

9:50 AM Discussion

10:00 AM Coffee break

10:25 AM *Symposium: Drosophila Neurobehavioral Genetics*
Chair and organizer: M. B. Sokolowski, University of Toronto

10:30 AM T. MacKay, North Carolina State University. The genetic architecture of complex behaviors in Drosophila

11:00 AM M. B. Sokolowski, University of Toronto. Variants as sources for genetic analysis of behaviour

11:30 AM R. Greenspan, The Neurosciences Institute, San Diego. Gene networks in Drosophila and the subtleties of behavior

12 Noon Lunch break

1:00 PM *Symposium: Genetics of Psychiatric Disorders Using Intermediate Phenotypes*
Chair and organizer: M. A. Enoch, National Institute on Alcohol Abuse and Alcoholism, Bethesda

1:05 PM D. Goldman, M. Radel, R. Vallejo, NIAAA. Alcoholism candidate atlleles and haplotypes in the GABA/serotonin domain

1:30 PM M. A. Enoch, K. White, C. Harris, K. Xu, J. Rohrbaugh, D. Goldman
NIAAA. EEG and ERP intermediate phenotypes for alcoholism and anxiety disorders

1:55 PM P. DePetrillo, NIAAA. Heart rate variability as an intermediate phenotype

2:20 PM M. Egan, T.E. Goldberg, J.C. Callicott, B.S. Kolachana, A. Bertolino, V.S. Mattay, R. Straub, D. Goldman, D.R. Weinberger, NIAAA. Intermediate

phenotypes demonstrate effects of COMT val158met and BDNF val66met on schizophrenia

2:45 PM *Paper session #1 Mouse Neurobehavioral Genetics*
Chair: L. Flaherty, Genomics Institute, Wadsworth Center

2:45 PM D. Brunner, T. Mullen, C. Leahy, D. Liu, Z. Sarnyai, A. Kottmann, PsychoGenics Inc. Effects of post-weaning social isolation and environmental enrichment on mouse behavior and gene expression

3:00 PM G.A. Carlson, J. Gilchrist, D. Peterson, D.A. Stephenson, and C. Toxopeus, McLaughlin Research Institute. Amyloid pecursor protein (APP) levels and a modifier locus on chromosome 7 affect penetrance of a new ENU-induced mutant allele of the voltage

3:15 PM S. Cabib and S. Puglisi-Allegra, Univ. "La Sapienza". Genetic regulation of neurobehavioral plasticity

3:30 PM W.E. Crusio, D.P. Wolfer, and H.P Lipp, Brudnick Neuropsychiatric Research Institute, Univ. of Massachusetts, and Institut für Anatomie, Universität Zürich. Knock-out mice: some simple experimental solutions to the genetic background and flanking gene problems

3:45 PM Snack break

4:15 PM *Symposium: Development of a Mouse Model of Intelligence: A Multiple Laboratory Approach*
Chair and organizer: R. Brown, Dalhousie University

4:20 PM D.A. Blizard, L. Cousino Klein, R. Cohen and G.E. McClearn, Pennsylvania State University. Mouse cognition: elimination of confounds

4:40 PM C. Locurto. Holy Cross College. The structure of individual differences in mouse (Mus musculus) intelligence: modular or general processes?

5:00 PM R. Brown and L. Stanford. Dalhousie University. Sex differences and individual differences in emotional responses, locomotor behaviour and spatial learning in HS mice

5:20 PM M.J. Galsworthy, J.L. Paya-Cano, L. Liu, C. Fernandes, L.C. Schalkwyk and R. Plomin. King's College London. An HS mouse model of general cognitive ability (g) for identifying quantitative trait loci (QTLs) and functional genomics

5:40 PM L.D. Matzel, Y. Han, and C.C. Gandhi. Rutgers University. Individual differences I n learning and synaptic function in CD-1 mice

Friday, November 9

8:30 AM *Posters - Grand Marina Room and Harbor Room* [**list at end of this document**]

1:00 PM *Symposium: History of Behavioral and Neural Genetics: Of Flies, Mice and Men* - Grand Marina Room
Chair and organizer: S. Maxson, Univ. of Connecticut

1:05 PM B. Ginsburg, Univ. of Connecticut. Fellow travellers on the road to the genetics of behavior: mice, rats & dogs

1:35 PM R. Greenspan, The Neurosciences Institute, San Diego. Genes and behavior in the fly: from natural variants to induced mutants and back again

2:05 PM E. Ginns, Brudnick Neuropsychiatric Research Inst., University of Massachusetts. Human behavioral genetics: making connections

2:35 PM Snack break

3:00 PM *Symposium: Elegant Genetic Analysis of Worm Behavior*
Chair and organizer: C. Rankin, Univ. of British Columbia

3:05 PM C. Rankin, J. Rose, and R. Norman, Univ. of British Columbia. The use of reporter genes to study the effects of experience on the anatomy of an identified synapse in the nematode *C. elegans*

3:35 PM B. Schafer, University of California at San Diego. Brain imaging and behavioral genetics in worms

4:05 PM K. Schaffner, George Washington Univ. *C. elegans* as a prototype for behavioral analysis: reductionistic, modular, and systems biology approaches

4:45 PM IBANGS Business Meeting - Grand Marina Room

7:00 PM Reception and cash bar

8:00 PM Gala Banquet

Saturday, November 10 - Grand Marina Room

8:30 AM *Paper session #2: Neurobehavioral genetics of flies and mice*
Chair: C. Janus, Univ. of Toronto

8:30 AM I. Dewachter, N. Caluwaerts, C. Kuipéri, C. Van den Haute, F. Van Leuven, Experimental Genetics Group, KU Leuven. Neuronal deficiency of Presenilin 1 prevents plaque formation but not behavioral defects of APP[V717I] transgenic mice

8:45 AM Y.S. Mineur, W.E. Crusio, Brudnick Neuropsychiatric Research Inst. Behavioral and neuroanatomical characterization of FVB/N inbred mice

9:00 AM D. Toma, K. White, R. Greenspan. The Neurosciences Institute, San Diego, and Yale University. Genetic basis for differential geotactic behavior in selected lines of *Drosophila melanogaster* analyzed with DNA microarrays and mutants

9:15 AM I. Ganguly, T.F.C. Mackay, R.R.H. Anholt, North Carolina State Univ. The Scribble protein is essential for olfactory avoidance behaviour in *Drosophila melanogaster*

9:30 AM V. Bolivar, M. Cook, L. Flaherty, Wadsworth Center. Mapping of quantitative trait loci for behavioral traits by the use of knockout/congenic strains

9:45 AM Coffee break

10:10 AM *Symposium: QTLs meet Mutagenesis*
Co-chairs and co-organizers, L. Tarantino, Novartis Research Foundation, and D. Goldowitz, Univ. of Tennessee

10:15 AM L. Tarantino, Novartis Research Foundation. QTLs and mutagenesis: past accomplishments and present challenges in complex trait genetics

10:40 AM W. Frankel, The Jackson Laboratory. QTLs meet mutagenesis in Bar Harbor

11:05 AM R. Williams, D.W Threadgill, K.F Manly, G.D Rosen, S. Shou, D.C Airey, J. Gu, L. Lu, Univ. of Tennessee. Integrated complex trait analysis of brain and behavior in mouse: near-term prospects for high-resolution QTL mapping using large RI and RIX sets

11:30 AM D. Goldowitz, Univ. of Tennessee. Regional mutagenesis - The practice and possibility of turning QTLs into QTgenes

11:50 AM Discussion

12 Noon Meeting Concludes

Presentation and Poster Abstracts

- in alphabetical order by presenter -

C57BL/6 and DBA/2 mice show strain specific gene-environment interactions on the elevated plus maze

Steve Adkins,1,2 Jordann Russell,4,5 Gilbert C. Wong,4 Margit Burmeister,1,2,3
1 Mental Health Research Institute, 2Department of Human Genetics, 3Department of Psychiatry, 4Summer Research Opportunity Program, University of Michigan, Ann Arbor, MI. 4Arizona State University, Dept. of Biology, Tempe, AZ

The Elevated Plus Maze (EPM), consisting or open and enclosed arms, was initially proposed as a screen for anxiolytics and subsequently became a commonly used tool to test anxiety in rodents. Some reports on the behavior of two commonly used mouse strains, C57BL/6 and DBA/2 on this maze are in sharp conflict. One lab reports that the DBA/2 strain spends much more time on the open arms than the C57BL/6 strain, while most labs report that the C57BL/6 strain spends more time on open arms then the DBA/2 strain. Here, we show how test environment influences behavior on this maze. Our results allow us to reconcile and explain the two discordant reports of EPM measures since we can reproduce BOTH results by changing the test environment. The EPM was modified to allow further dissection of test parameters. We hide visual aspects of the test environment (3 conditions) and use two different rooms to conduct tests: 6 environments. Range measures, 'open ends enter. and ranged to an open end' were more discriminating than the traditionally used 'time on open arms' measure. Aspects of the test environment, test room and test condition differentially affected the behavior of the mouse strains contributing to a significant strain difference ($p < 1x10\text{-}4$, $n = 7, 8$) on a modified EPM. Using such range measures while examining environmental features that differentially affect strains may lead to more reproducible performances on the EPM and a wider understanding of the approach-avoidance conflict evidenced by the EPM.
This work was supported by the National Institutes of Health, NS32130 to MB, TG-MH15794 to SA, and the UM Summer Research Opportunity Program to JR and GW.

Genes Involved in LTP, excitability, Place Cell firing & learning and memory

D. Bai, F.Taverna, Y.M. Lu, J. Yan, W.Y. Lu, J. Roder
Samuel Lunenfeld Research Institute at Mount Sinai Hospital, Mt. Sinai Hospital, 600 University Avenue, Toronto Ontario Canada M5G 1X5

We have used reverse genetics to probe the relationship between LTP and learning and memory in the hippocampus. Mice carrying a null mutation in MGluR5 showed a specific deficit in performance on the Morris water maze and context-dependent fear conditioning but normal control behaviors. In addition, hippocampal slices show a total loss of LTP of the NMDAR-EPSC. From rescue experiments, mGluR5 is coupled through PFC, pyk-2 (cakb) and src to the NMD/LTP is also accompanied by an increased coupling of the EPSP to the firing spike (E-S). Blockade of the Ca2+ -sensitive phosphatase, calcineurin, prevents induction of E-S coupling by inducing a long-lasting depression (LTD) of the GABAA - mediated inhibitory postsynaptic potentials (IPSPs). This LTD of the IPSP was prevented by blockade of NMDA receptors. Thus, the tetanus that elicits NMD dependent LTP mediates a coordinately regulated double function. It produces LTP of the EPSP and, concomitantly, LTD of the IPSP that leads to enhancement of E-S coupling. LTP normally appears limited and saturated, but our GluR2 null mutants had very high levels that did not saturate. Recent experiments suggest there is normally negative feedback between the AMPAR and NMDAR. This non-Hebbian LTP was associated with impaired place cell activity. Finally, we are using a forward genetic screen of ENU treated mice for new learning and memory mutants.

Larval foraging behaviour in the fruit fly Drosophila melanogaster

A. Belay, M. Sokolowski
Department of Biology, University of Toronto at Mississauga, Ontario, Canada

Allelic variation in the foraging (for) gene of the fruit fly Drosophila melanogaster accounts for naturally occurring rover (forR) and sitter (fors) larval food search behaviours. In a foraging environment, rovers exhibit longer foraging pathlength than sitters. This foraging trail difference is not exhibited in the absence of food. Cloning of the for gene demonstrated that it is synonymous with dg2, which encodes a Drosophila cGMP-dependent protein kinase (PKG). for is a complex gene with three alternatively spliced major transcripts (T1, T2, and T3). Rovers express higher levels of for transcripts and show a higher PKG enzyme activity than sitters. These findings suggest a role for PKG mediated signaling in larval foraging behaviour. When and where PKG functions to generate this natural variation in Drosophila foraging behaviour remains to be investigated. T2 cDNA under the control of a leaky heat shock promote in transgenic sitter rescued the sitter behaviour to rover like, suggesting that T2 may be involved in larval foraging behaviour. Targeted expression studies are on the way to investigate where PKG functions to produce the rover/sitter behaviours. In situ hybridization studies showed that T2, for example, is expressed in the larval brain as well as the developing adult visual system. We have also localized a number of transposable element enhancer> trap insertions within for. All of them localize near T2 and show identical expression patterns in the CNS, which corresponds in part to T2 expression, found in the in situs. Further more, these P-element inserts in for are pupal-lethal suggesting that for is a vital gene.

Gaba-A Alpha1 or Beta2 Knockout mice display different behavioral phenotypes

Blednov YA, Whiting PJ, Rosahl T, Wallace D, Harris RA
Waggoner Center, University of Texas at Austin, TX 78746, USA and Neuroscience Research Center, Merck Sharp and Dohme Research Laboratories, Harlow, Essex, CM20 2QR, United Kingdom. Institute for Cellular and Molecular Biology University of Texas Molecular Biology Building, Room 1.124 Austin, TX

The Gaba ergic system is the major contributor of the inhibitory tone throughout the CNS. Gaba-A receptors are the site of action of a number of clinically important drugs, producing anxiolytic, sedative, myorelaxant, anticonvulsant and anesthetic effects. We addressed the physiological role of two the most abundant subunits of Gaba-A receptor Alpha1 or Beta2 by studying the behavioral phenotypes of homozygous null mutant mice. The Beta2 animals demonstrated normal initial (first 20 min) motor activity in the home cage but markedly slower habituation to novelty. The initial (first 2.5 min) motor in an open-field was similar in mutant and control mice, but during the followed five 2.5min sessions (habituation phase) the Beta2 mice were more active, mainly in the central area, than wild type animals. Some signs of less anxious behavior were also found in Beta2 knockout mice in elevated plus-maze (more time in open arms and higher number of unprotected head dips and stretches) and the canopy test (higher percent of time spent in the exposed area). In contrast, Alpha1 null mutant mice were hypoactive in the home cage (initially as well as in the open field test mainly in th central area. In the elevated plus-maze test Alpha1 knockout mice showed less time and a lower percentage of entries into the open arms, and fewer unprotected head dips and stretches). In the igrave canopy test, these mutant mice showed the less time spent in the exposed area higher number of protected but lower number of unprotected stretches in comparison with WT mice. These data demonstrate that deletion of alpha1 or beta2 subunits of Gaba-A receptor leads to different behavioral phenotypes despite their similar localization and the co-assembly of these subunits in the brain.

Supported by NIAAA.

Mouse cognition: elimination of confounds

David A. Blizard, Laura Cousino Klein, Rachel Cohen and Gerald E. McClearn
Pennsylvania State University

Individual differences in performance in tests of cognition may reflect a variety of sensory and motivational processes. We will describe the development of a test that attempts to minimize stress and does not employ organic deficits to motivate performance.

Mapping Quantitative Trait Loci for Intersession Habituation with BXD Recombinant Inbred Mice

V.J. Bolivar, C.J. Bennett, and L. Flaherty
Genomics Institute, Wadsworth Center, Albany, NY, 12201-2002

All though behavioral habituation to a novel environment has been studies in rodents for decades, little attention has been given to understanding the role of genetics in this process. Using 5-minute exposures to a dark activity monitor, we recently reported inbred strain variability in intersession habituation performance (V. J. Bolivar, B. J. Caldarone, A. A. Reilly and L. Flaherty, 2000, Behav. Genet. 30, 285-293). Of the inbred strains tested, two of the most divergent were C57BL/6J (B6) and DBA/2J (D2). B6 mice displayed intersession habituation, but D2 mice did not. Based on the difference in performance between the two inbred strains, we selected the BXD recombinant inbred series for our QTL analysis. A total of 25 BXD strains (19-20 males per strain) were tested for habituation performance in a dark activity monitor over 3 days. A highly significant QTL (permutation test, genome scan corrected, $p<.01$) was detected on Chromosome 15. This QTL is independent of other QTLs found for baseline activity in our study. The RI-generated region on Chromosome 15 was subsequently confirmed by analysis of a B6D2F2 population. We are currently in the process of fine mapping this region and hope to be testing candidate genes shortly.

This work was supported by NIH Grant MH58599.

Comparison of the behavioral effects of MK-801 and apomorphine on C57BL/6, BALB/C, DBA, and SVEV129 mice

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Schizophrenia, a disease which strikes approximately 1% of the world's population regardless of ethnicity, has a familial component. In an effort to examine what may be alternate ways of modeling the etiology of schizophrenic symptoms, we investigated the effects of a dopamine agonist, apomorphine, and an antagonist for the glutamate NMDA receptor, MK-801. We have previously reported that there is a difference in the neuropathology which results from medium to large doses (1 to 10 mgs per kg) of MK-801 in six inbred and two outbred strains of mice. Here we report behavioral results of administration of .1, .3, and 1 mg/kg of MK-801 and 1 mg/kg of apomorphine in four inbred strains of mice: C57BL/6 (B6), BALB/c, DBA, and SVEV129. Comparisons were made of climbing behavior, activity as measured by beam breaks in an automated monitor, and many stereotypical behaviors. The results included significant differences between strains in measures of activity due to strain and treatment, with SVEV129 recording the lowest activity scores of all strains on all treatments. There were significant differences on all measures of climbing due to strain, but treatment accounted for differences only in episodes of climbing. Strain by treatment interaction accounted for differences in sniffing, mouthing, headweaving, forepaw treading, some measures of grooming, and rearing. Briefly,

these data suggest that the effect of the treatments may be very different depending on the strain, and presumably the genetic makeup, of the mouse.

Sex differences and individual differences in emotional responses, locomotor behaviour and spatial learning in HS mice

Richard Brown and Lianne Stanford
Dalhousie University

We have developed a test battery for measuring emotional responses, locomotor behaviour and learning and memory in mice. This test battery ("Mouse IQ") is based on the concept of multiple memory systems in the brain and uses tests that involve th hippocampus, cerebellum, amygdala, striatum, and cortical memory systems. We will present a subset of data from a larger study of sex and individual differences in the behaviour of HS mice which is in progress in our lab; Data from tests on the elevated plus maze, open field and rotarod, spontaneous alternation in a T-maze, and spatial learning in the Barnes maze, Morris Water maze and Hebb-Williams maze will be reviewed; Individual differences in patterns of behaviour were analyzed within and between tests using correlational analyses. We will use our results to discuss the advantages and disadvantages of using large test batteries to assess mouse behaviour.

Effects of post-weaning social isolation and environmental enrichment on mouse behavior and gene expression

Dani Brunner, Tanner Mullen, Christina Leahy, Dong Liu, Zoltan Sarnyai, Andreas Kottmann

Post-weaning rearing condition such as social isolation (IS) or environmental enrichment (EE) has profound impact on mammalian brain function. Studies show that IS is associated with increased anxiety and aggression, vulnerability to substance addiction, and impaired cognitive performance, whereas EE is associated with reduced anxiety, improved learning and memory, resilience to neural toxicity insults, and speedy recovery from brain injury. Such effects are permanent, persisting throughout life, and thought to influence the development of neuropathology that exists in a number of psychiatric disorders. To further investigate the neural mechanisms underlying these early life experience initiated long-term changes, we have applied a newly developed, state of art PsychoScreen testing scheme to systemically profile the effects of IS or EE in mice. The data revealed differences in behavior as a result of different early life experience, suggesting according changes may occur in the neural systems that regulate emotion, cognition and other processes. In parallel, we are examining the effects of IS or EE at molecular level to identify genes which may be differentially regulated in mediating the environmental effects on behavior. Our finding indicates that IS may be used as a potential behavioral model to study mental disorders and provide useful insights in discovery of novel therapeutic approach to treat such illnesses.

Genetic regulation of stress-induced neurobehavioral plasticity

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Plasticity of neurobehavioral phenotypes in mature mice of the inbred strains C57BL/6 and DBA/2 was investigated. Strain differences for behavioral responses to amphetamine challenge and for emotional reactivity to novelty were abolished or reverted.

The use of naturalistic-types olfactory stimuli (specialised antipredatory cues emitted by the Striped millipede Ommatoiulus sabulosus) for detecting and screening sensory competencies of adult mice

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Neurosciences often forget basic Darwinism, as criticised e.g. in the Introduction of "Behavioural Brain Research in Naturalistic and Semi-Naturalistic Settings: Possibilities and Perspectives" (Alleva E., Fasolo A., Lipp H.P., Nadel L., and Ricceri L., Dordrecht: Kluwer Academic Publishers, 1995). In order to set up a novel and ethologically-relevant methodology, friendly exploitable when studying olfactory capabilities of transgenic mice, we characterised and analysed the behavioural responses of sexually-mature male and female outbred CD-1 mice individually exposed to a Stimulus Object (SO) consisting of either (I) a Striped millipede [Ommatoiulus sabulosus (L.)], a very common Myriapod species that specialised in repulsive and persistent odour in presence of a predator or (II) a larva of the lepidopteran Greater wax-moth [Galleria mellonella (L.)], closely resembling the millipede in shape and dimensions but which does not secrete a repulsive defensive odour cue. Avoidance and non-avoidance behavioural responses were measured for 10 min for 3 consecutive days. Millipede exposure induced in both sexes a dramatic increase in the avoidance-type "Digging". Moreover, animals exposed to the millipede were repelled, contrary to what observed in those exposed to a wax-moth; this difference was evident in the Eating behaviour, totally absent in mice exposed to the millipede. Sex differences emerged only for Locomotion, females appearing more active than males. In a second series of experiments, adult male and female CD-1 mice were exposed to a SO consisting in a millipede-shaped sponge previously soaked either (III) in a distilled water-solution (100ml) containing 5g of Toluquinone (Fluka: a single chemical component of the exudate secreted by the millipede), or (IV) in distilled water. The behaviours performed by mice when exposed to the SO were scored for 15 min for 5 consecutive days. Moreover, performances in a hot-plate test (50±0.5 C) were assessed immediately after a 15-min SO exposure, since pain reactivity is known to be affected by naturalistic-type olfactory stimuli such as those emitted by rodent predators such as foxes, cats, weasels, etc. Toluquinone exposure affected Digging and Stretch Back Sniffing, suppressed nearly completely Catching and Eating the SO and generally increased arousal of these mice, particularly in females. Moreover Toluquinone-exposed mice showed a subtle yet significant decrease of pain threshold. Since transgenic or genetically-modified mice could suffer from subtle, hyperosmia, or hyposmia or anosmia as a side-effect of genetic manipulation, we strongly recommend Toluquinone exposure as a new, phylogenetically robust, easy accessible and low-cost method for screening sensory/olfactory competencies of adult mice.

This work was supported by intramural ISS funding and ISS-Comune di Roma project "A pilot study on urban consistency of mouse population for characterising 'sentinel' species for environmental neurotoxicants" to E. A.

Amyloid Precursor Protein (APP) Levels and a modifier locus on Chromosome 7Affect Penetrance of a New ENU-Induced Mutant Allele of theVoltage Dependent Calcium Channel Gene Cacna1a

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Amyloid plaques in the brains ofAlzheimer's disease (AD) patients are composed of Apeptide cleavage products of APP. Although APP is intimately involved in AD, its physiological functions and precise role in disease are unknown. A sensitized screen for ENU-induced mutations in genes relevant to APP function was undertaken. One of the 17 dominant mutations recovered

from screening 1,971 mice was affected by APP expression demonstrating the feasibility of sensitized screens for induced mutations in mammals. Offspring of ENU-treated C57BL/6J (B6) males and FVB/N (FVB) were screened at weaning and again two weeks later using simple battery of neurological and behavioral tests. A G1 female with a subtle highstepping gait was detected and was mated to FVB/N and FVB.129-App-null mice to test heritability. Twenty-six of 69 offspring presented around the time of weaning with phenotypes that were not seen in the founder. The affected mice exhibited poor coordination and exaggerated, slow movements. This severe phenotype disappeared within 3 days of weaning and only a mild gait abnormality persisted. The mutation, designated Highstepper or Hst, was mapped to a region of Chromosome 8 in the vicinity of Cacna1a. Allelism tests with B6.D2-Cacna1a-tg (tottering) mice indicated that Hst is a new mutant allele of Cacna1a. Interestingly, the behavior of Hst/tg offspring from the allelism test cross could not be distinguished that of wild type homozygous littermates, in contrast the semi-dominance of Hst when crossed to FVB. This suggests that dominance of Cacna1a-Hst is dependent on genetic background. A genome scan of affected and non-affected carriers of the Hst mutation suggests a modifier of penetrance on distal Chr 7. Results from genotyped Hst carriers also indicated an effect of APP concentration on penetrance. Only 36 of 58 (62%) Hst carriers homozygous for wildtype App were affected, while 43 of 57 (75%) carriers with one App null allele were affected. In contrast, all 17 App null homozygous carriers of Hst exhibited the phenotype.
This work was supported by grants from the American Health Assistance Foundation and the National Institute on Aging, USPHS.

Some simple experimental solutions to the genetic background and flanking gene problems
Wim E. Crusio,1 David P. Wolfer,2 Hans-Peter Lipp,2
Knock-out mice:1Brudnick Neuropsychiatric Research Institute, Dept. of Psychiatry, University of Massachusetts Medical School, Worcester, MA 01604, USA. 2Institut Anatomie, University of Zurich, Zurich, Switzerland

In model organisms, inducing null mutations by means of homologous recombination provides a powerful technique to investigate gene function that has found wide application in many different fields. However, it was realized some time ago by Gerlai (TINS 19: 177-181, 1996) that the specific way in which such knockout mutants are induced leads to an experimental confound, making it impossible to separate the effects of the induced null mutation from those of alleles originating from the embryonic stem cell donor. In addition, effects from null mutations may be altered on different genetic backgrounds. Here we present some simple experimental solutions to test for flanking allele effects, simultaneously providing a first test of interaction between the mutation and the genetic background.

Heart Rate Variability as an Intermediate Phenotype
P. Depetrillo - NIAAA

Accumulating evidence strongly suggests that several measures of heart rate variability are heritable and may be used for stratifying populations into sub-groups, thereby assisting the search for genetic influences underlying autonomic function and behavior. Both time-domain and frequency-domain methods have been employed with some success to derive these parameters. However, these methods introduce technique-specific variance to the analysis due to the autocorrelated nature of the cardiac interbeat interval signal. Non-linear analysis techniques for extracting parameters of heart rate variability that circumvent these limitations will be discussed, and the results of applying these techniques to cardiac interbeat interval time-series obtained from several different population groups will be reported.

Neuronal deficiency of Presenilin 1 prevents plaque formation but not behavioral defects of APP [v7171] transgenic mice

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Experimental Genetics Group (LEGT_EGG), K.U.Leuven, B-3000 Leuven, Belgium

Major therapeutic strategies for AD aim to decrease the production of amyloid peptides by inhibition of - or (-secretases. Presenilins are polytopic transmembrane proteins that are essential for development and for amyloid peptide formation by (-secretase activity (De Strooper et al, Nature, 1998, 391: 387-390). Using loxP/Cre-recombinase mediated deletion, we have now generated adult mice that are specifically deficient in PS1 in their central neurons, denoted as PS1(n-/-). To that end we generated transgenic mice expressing Cre-recombinase specifically in neurons (mouse thy1-promoter) and its postnatal expression circumvented developmental problems. PS1(n-/-) mice were viable and fertile, with normal brain morphology, as well as normal behavior and cognition. This demonstrates that PS1 is not needed in brain of adult PS1(n-/-) mice, while endogenous amyloid peptides decreased about 8-fold, with accumulation of APP -C-stubs, their obligate precursors. In APP[V717I] x PS1(n-/-) triple transgenic mice amyloid pathology was prevented in mice as old as 18 months, in sharp contrast with the parent single APP[V717I] transgenic mice that all develop amyloid pathology from the age of 10 months onwards (Moechars et al, J Biol Chem, 1999, 274: 6483-6492; Van Dorpe et al, Am. J. Pathol, 2000, 157: 1283-1298, Dewachter et al, J Neurosci, 2000, 20: 6452-6458; Schneider et al, J Biol Chem, 2001, 276: 11539-11544). Despite the expected biochemical and pathological correction of amyloid peptide levels and of the amyloid pathology, neophobia and non-spatial memory remained impaired and was even worsened. These results are important for our understanding of the function of PS1 and for the pathogenesis of AD, as well as for therapeutic strategies aimed at decreasing secretase activity.
Supported by EEC-Biotech, EEC-5FP, FWO-Vlaanderen, IUAP-IV, KULeuvenR&D.

Down syndrome: Abnormal maturation of cortical circuitry in the Ts65Dn trisomic mouse model

R. Benavides-Piccione,1 G. N. Elston,1,2 J. DeFelipe,1 **M. Dierssen**,3 J. Florez,4
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Positive short-term effects of early intervention when treating children with Down syndrome (DS) are well recognized. Replicating this approach in experimental models of this disease offers great advantages for the study of multifactorial techniques such as environment enrichment (EE). In the present study we investigated how EE affects the maturation of neo-cortical circuits in the TS65Dn DS mouse model. Ts65Dn and control littermates were raised in either standard or enriched environments. At one year of age, the animals were sacrificed by injection of pentobarbitone sodium and perfused with 4% paraformaldehyde in phosphate buffer (0.1 mol/l). Pyramidal cells were studied in layer III of the left frontal pole of 2 animals in each of the four groups. Cells were intracellularly injected with Lucifer Yellow in flat-mounted cortical slices. All experiments were performed according to the NIH guidelines on animal welfare. We found that the effects of EE were different between control and DS animals. EE resulted in a significant increase in the number of branches and spines in the dendritic arbors of cells of control animals, suggesting increased integrative ability and functional complexity. However, EE did not affect the morphological characteristics of pyramidal cells sampled from DS animals. Thus, the changes in cortical circuitry induced by environment enrichment in control animals, that are not present in DS mice, suggest that brain disturbances secondary to this chromosomal pathology may affect severely the mechanisms underlying cerebral development. As cortical circuitry may

be determined by genetic and epigenetic factors, further studies are required during development to establish how these features interact.
Supported by Lejeune Foundation and FIS

Functional Genomics of Memory in Drosophila
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Three features of long-term memory are conserved across animal phyla. First, memories are initially stored in a short-term labile form but can progress to a longer lasting, stable form. Second, long-term but not short-term memory requires a new program of gene expression. And third, many tasks require repeated training sessions interspersed with rest intervals (spaced training), rather than repeated training without rest intervals (massed training), to produce long-term memory.
In Drosophila, spaced training results in several short-term forms of memory, as well as in long-term memory, which is CREB- and protein synthesis-dependent. In contrast, memory after massed training is less stable, CREB independent, and insensitive to protein synthesis inhibitors.We have used these behaviorally specific training protocols and mutations that disrupt memory, in combination with Affymetrix gene chips, to characterize a genomic response to memory formation.We have identified novel genetic pathways involved in memory consolidation.
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Intermediate phenotypes demonstrate effects of COMT val158met and BDNF val66met on schizophrenia
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Patients with schizophrenia have a variety of neurobiological abnormalities referable to the prefrontal cortex and mesial temporal lobe. These include cognitive deficits, alterations in prefrontal physiology seen with fMRI, and reduced measures of hippocampal n-acetylaspartate (NAA), an index of neuronal integrity seen with MRSI. To assess the suitability of these measures for use as intermediate phenotypes, we examined a cohort of over 225 probands, 300 sibs, and 150 controls. Siblings performed markedly worse than controls on measures of working and verbal memory. Relative risk for impaired performance ranged from 1.8 to 3.8. Using fMRI during a working memory task, siblings, similar to probands, demonstrated a pattern of inefficiency; compared to controls. Hippocampal NAA measures also showed a slight reduction in siblings. These phenotypic measures were used to examine the effects of nonconservative SNPs in two genes known to affect prefrontal and hippocampal function, COMT and BDNF, respectively. COMT, which appears to regulate specifically prefrontal dopamine, has a common functional variant (val158met) that produces a fourfold change in enzyme activity. In all groups, the number of low activity met alleles was correlated with superior performance on prefrontal cognitive measures and fMRI measures of efficiency. Using the transmission disequilibrium test (TDT), the val allele was preferentially transmitted from parents to their schizophrenic offspring, demonstrating both linkage and association. BDNF val66met genotype, a neurotrophin involved in LTP and memory, was associated with performance on measures of verbal memory, with met/met homozygotes performing worse than other genotype groups. BDNF genotype also accounted for 2% of the variance in hippocampal NAA measures, with met/met homozygotes again showing reductions relative to the other genotype groups. We

did not find an excess transmission of the meallele in over 200 trios. These data demonstrate that several intermediate phenotypes may be useful in genetic studies of schizophrenia. Furthermore, COMT appears to exert a slight effect on risk for schizophrenia by virtue of its effects on prefrontal physiology. In contrast, BDNF appears to affect normal human verbal memory, but does not appear to affect risk for schizophrenia.

EEG and ERP intermediate phenotypes for alcoholism and anxiety disorders

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Alcoholism and anxiety disorders are heritable, common, complex conditions. The inherited causative neurobiology is still relatively unknown. One approach that is likely to increase power to find vulnerability genes is to utilize descriptors that are less genetically complex than clinical diagnosis, such as dimensional or quantitative measures (for example, neuroticism or harm avoidance for anxiety) and associated biological traits, including intermediate phenotypes, whose origins may be closer to gene action. Two pertinent phenotypes for alcoholism and anxiety disorders - potentially reflecting intermediate mechanisms in the brain are the low voltage alpha resting EEG trait (LVA) and the P300 event related potential (ERP). Alpha is the predominant EEG waveform at rest. LVA individuals (8-12% of the general population) have a distinctive EEG with no (or very scanty) alpha. The P300 ERP is a scalp-recorded voltage change elicited in response to infrequent, unpredictable, target stimuli occurring among frequent nontarget stimuli. A robust finding is that P300 amplitude is reduced in alcoholics (Porjesz & Begleiter 1998). In studies on 247 Caucasians and 365 Plains Indians ascertained from the community, we have found that alcoholism comorbid with anxiety disorders is associated with both LVA and reduced amplitude P300 ERP (Enoch et al 1995, 1999, 2001). These studies will be reviewed. We have begun to use these EEG and ERP intermediate phenotypes in candidate gene analysis. A prime candidate for alcoholism and anxiety vulnerability is the gene for catechol-O-methyltransferase (COMT), a major enzyme involved in the metabolism of dopamine and norepinephrine in the CNS. We found that the low activity Met allele of the COMT Val158Met functional polymorphism (and particularly the Met/Met genotype) was associated both with LVA and increased harm avoidance in Caucasian and Plains Indian women. Further candidate gene analyses and linkage analyses are planned.

Vanaso is a quantitative trait locus for olfactory behavior in Drosophila

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Odor-guided behavior is essential for the survival and reproduction of most organisms. Although considerable progress has been made in elucidating the genes and mechanisms that underlie olfaction, we do not know what genes affect naturally occurring variation in olfactory behavior. We utilized Drosophila melanogaster as a model system to study quantitative variation in odor-guided behavior using a "dipstick" assay to quantify the behavioral response to the repellent odorant, benzaldehyde. There was highly significant ($p<0.0001$) genetic variation for olfactory behavior in a population of 98 recombinant lines derived from Oregon and 2b Drosophila strains. Composite interval mapping was used to map quantitative trait loci (QTL) for olfactory behavior by linkage to polymorphic molecular markers. A single QTL with effects in both sexes was mapped to the third chromosome at cytological position 61A-66B. High-resolution quantitative deficiency complementation tests showed that there were two sex-specific QTL

affecting odor-guided behavior in this region: a female-specific QTL at 62B1-9 and a male-specific QTL at 66B2-8. Quantitative complementation tests with all available mutant stocks in these regions revealed that the lethal P element insertion in l(3) 04276 (62 B4-5) failed to complement the female-specific QTL. This gene, which we have called "vanaso", encodes a novel protein with a GTP binding motif, which is expressed in the main olfactory organ, the third antennal segment. This is one of few cases in which a gene corresponding to a QTL has been identified, and the first description of a gene affecting sex-specific genetic variation in olfactory behavior.
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QTL genes for fearfulness in the Roman rats

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In searching quantitative trait loci (QTLs) for anxious temperament (fearfulness), an F2 intercross of nine hundred rats derived from one of the best existing rodent models of anxiety (the Roman high- and low-avoidance rat strains) was phenotyped and genotyped. Fearful behavior was measured by means of a test battery encompasing tests of unconditioned and conditioned responding to fear-inducing stimuli. Anxious profile was characterized, as shown by factor analytic techniques, by three simple principal factors which distinguished learned from unlearned fear. Multivariate analyses of the behavioral/genetic data revealed that one QTL, on rat chromosome 5 (D5Rat100-D5Wox4), influenced conditioned and unconditioned fearful behavior in a way that parallels the effects of anxiolytic drugs.There seemed to be a partial fit between the factor analytic map of fearfulness and the QTL candidates. Thus, all the measures loading on the first factor (Learned Fear) were linked to the QTL on chromosome 5, whereas measures of unconditioned fear loading onto factors 2 and 3 ("Emotional Reactivity" and "Fear of Heights") were associated to that QTL plus a variety of other QTL candidates. The fact that a consistent cluster of fear-induced responses were bound up with a single QTL on Chr5, the direction of effects of the QTL alleles, and the coincidence between the behavioral profiles of anxiolytic drug and genetic action, are consistent with the QTL containing at least one gene with a pleiotropic action on anxiety.
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Mapping of quantitative trait loci for behavioral traits by the use of knockout/congenic strains

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We have recently explored the use of knockout/congenic mouse strains for isolating and mapping quantitative trait loci (QTLs) that influence behavior. Since most knockout strains are also congenic strains that have retained a short piece of 129 genetic material, flanking the target ablated locus andderived from 129 embryonic stem cells, they can also be used to test for

polymorphisms surrounding the target locus. There are over 50 of these knockout/congenic strains commercially available and represent a unique source for mapping of genes influencing behavioral traits. To investigate the utility of this resource, we have used these strains to identify regions that affect certain behaviors in mice, including open field activity. We have also developed a testing scheme to enable the separation of flanking and target gene effects on behavioral performance. In our screens for open field activity, we have found that at least two knockout/congenic strains, B6-IL10! and B6-Cd28-/-, behave differently than B6. In further testing with B6-IL10-/-, we have found that flanking 129 genetic material (surrounding the target locus) is responsible for the different behavior of these mice. These observations are in accord with the work of others that map a QTL for open field activity to chr. 1. This scheme has several distinct advantages to more conventional protocols since it yields a ready-made congenic strain as well as allows repeated observations of genetically similar mice that increase the power of the investigation.

Characterisation of motion sickness in the mouse

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Motion sickness (MS) is an illness triggered by a ìsensory conflictî involving the vestibular system, occurring when sensory inputs regarding body position in space are contradictory or different from those predicted from experience. In a large variety of animal species MS is associated with the occurrence of an emetic response. In rat, a species incapable of emetic response, pica behaviour (the consumption of non-nutritive substances, such as kaolin, sawdust and wood) has been considered an appropriate index of MS. Our studies were aimed to characterise MS in CD-1 mice and to evaluate possible age-related differences in the susceptibility to this syndrome during late postnatal development in this rodent specie. The occurrence of MS was evaluated in both male and female mice on postnatal day (PND) 28, 42, 60 and at adulthood. The animals were exposed to a rotation-induced change in the vestibular inputs using a custom-made centrifuge device. Pica behaviour, measured through kaolin consumption, and ethological-type scoring of different activities were used to evaluate the behavioural response before, during, and after exposure. Moreover, in order to correlate behavioural changes with alteration in central levels of neurotrophins, brain levels of Nerve Growth Factor (NGF) and Brain Derived Nerurotrophic Factor (BDNF) were assessed following 1h of rotation.

An HS mouse model of general cognitive ability (g) for identifying quantitative trait loci (QTLs) and functional genomics

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In our first studies of heterogeneous stock (HS) mice, we found that diverse cognitive tasks intercorrelate, suggesting the presence of a general cognitive ability (g). We attempted to control motivation-related confounds by employing a battery that was cross-motivational. Furthermore the commonality across cognitive tasks was robust to regression by sex,anxiety measures and removal of outliers. Our ongoing work aims to greatly increase the sample size in order to confirm these preliminary results and to begin to explore genetic correlates of cognition. Our QTL strategy will focus on functional polymorphisms in human and mouse candidate genes. Initially all HS mice tested will be genotyped individually but when the sample is sufficiently large

we will use DNA pooling of the extremes in order to be able to screen thousands of DNA markers for associations with g.

The Scribble protein is essential for olfactory avoidance behaviour in Drosophila melanogaster

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We have used P[lArB]-element insertional mutagenesis to identify genes that contribute to olfactory behavior in Drosophila melanogaster. Disruption of the scribble gene results in impaired responsiveness to benzaldehyde and other repellant odorants. Adult smell-impaired mutants (smi97B) show a sexually dimorphic phenotype with stronger smell-impairment in females than in males. Mutant larvae display decreased chemotactic responses to amylacetate and benzaldehyde. Precise excision of the P[lArB]-element restores wild-type olfactory behavior. A deletion line generated by an imprecise excision of the P-element displays a phenotype in which females behave normally, but males are severely smell-impaired. In smi97B flies the P[lArB]-element has inserted about 1kb upstream from the transcription initiation site of the scribble gene. Transgenic expression of a scribble cDNA clone in the smi97B background, rescues the mutant smell-impaired phenotype. Complementation studies reveal that deficiency line Df(3R)Tl-X (97B; 97D1-2) and null alleles of scribble, scrib1 and scrib2 fail to complement smi97B. However, only female-specific failure to complement is observed with the scribble hypomorph, scribS042405, while inter-allelic complementation is observed with the hypomorphic allele scrib(j7B3). This suggests the presence of alternatively spliced variants. Northern analyses confirm these results and reveal sex-biased transcripts that may give rise to the sexually dimorphic phenotype observed behaviorally. Western blot analyses corroborate these sex-specific expression patterns. In situ hybridization and immunohistochemical localization of the Scribble protein reveal expression throughout the CNS with enrichment of the gene product in the third antennal segment, the antennal nerve, and the lateral protocerebrum. Scribble contains 16 leucine-rich repeats and 2-4 PDZ domains thought to mediate protein-protein interactions. Bilder and Perrimon previously localized Scribble to septate junctions in embryos, where it is responsible for sequestering apical polarity determinants. The smi97B line is the first homozygous viable mutant allele of scribble and provides us with both larval and adult phenotypes that can be studied. Based on its similarity to other synaptic proteins and its localization pattern, we speculate that Scribble plays a role in synaptic organization and is essential for relay of chemosensory information in the CNS.
This work was supported by the W. M. Keck Foundation and grants from the National Institutes of Health.

Behavioral and immunocytochemical characterization of C57BL/6 Tac TgN(APPV717F) mice

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Alzheimer's disease (AD) is one of the most common forms of dementia among the elderly. Transgenic mice which overproduce a mutated form of human amyloid precursor protein (APP) have been used as an animal model of AD. Mice of mixed genetic background (C57BL/6, DBA/2J, Swiss Webster) with the mutation VÆF at position 717 (APPV717F) exhibit age- and region-dependent amyloid plaque deposition and cognitive impairment. Commonly used APP Tg mice derived from various hybrid backgrounds may exhibit substantial phenotypic variation

within and between Tg lines. Furthermore, the breeding schemes used to maintain the Tg lines often makes the choice of suitable wildtype controls problematic. In an attempt to address these problems, the APPV717F Tg mice were backcrossed to the C57BL/6 strain for over 14 generations, resulting in C57BL/6 Tac-TgN(APPV717F) mice that are hemizygous for the APP transgene. Behavioral testing revealed impairments in cognition as measured in a mouse spatial learning holeboard assay. Transgenic mice had deficits in working memory, reference memory and an overall increase in total errors compared to age-matched C57BL/6 mice. Immunocytochemical analysis of a subset of mice demonstrated robust age- and region-dependent Ab deposition similar to previously characterized APPV717F hybrid mice. These congenic mice may be used to more precisely characterize the effects of various agents in altering the progression of AD-like pathology.
This work was supported by Eli Lilly and Co.

Force transducer based movement detection in fear conditioning in mice: A comparative analysis

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Fear conditioning (FC) allows the dissociation of hippocampal and non-hippocampal behavioral function in rodents, and has become a diagnostic tool in transgenic mouse research employed to investigate mutation induced changes in brain function. Although the procedural details of the paradigm have been established, quantification of the behavioral output, freezing, remains problematic in mice. Observation based techniques are time consuming and may be subject to bias, while movement detection with photocells is imprecise. Here we describe an alternative method for movement detection based on an electronic force transducer system that allows the quantification of acceleration forces generated by a moving subject. We compare behavior of two inbred strains of mice (C57BL/6 and DBA/2) whose performance is known to differ in hippocampal tasks including FC. The comparison is made using multiple techniques: the force transducer approach, and three observation based methods, a computer aided event-recording approach, a traditional time sampling paper/pencil method, and a subjective impression based scoring system. In addition, we investigate the correlation structures of behavioral elements quantified by event recording using Principal Component Analyses and conclude that fear may manifest in multiple forms and in a stimulus and genotype dependent manner. We suggest that the force transducer system provides precise quantification of movements in an automated manner and will allow high throughput screening for mutation and drug effects in mice. However, we also argue that fear responses can be complex and freezing behavior may not be the only measure of fear or fear associated memory.

Behavioural assessment of mice lacking the adenosine A1 receptor

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We have recently shown that mice with a targeted disruption of the second encoding exon of A1receptor (A1R) bred and gained weight normally and had a normal heart rate, blood pressure, and body temperature. In most of thebehavioural tests they were similar to A1R+/+ mice, but A1R-/- mice showed signs of increasedanxiety and termal hyperalgesia. Decreased

hypoxic neuroprotection was also observed. Here we present the complete behavioral assessment of these mice with new results on motor performance (traction, motor coordination and muscular resistance), circadian activity (in activity cages), exploratory activity (open-field and hole-board tests), anxiety/emotionality (plus-maze, dark and light box tests), aggressivity (resident-intruder test) and learning and memory (several Morris water maze tasks: place learning, removal, reversal, cue learning and working memory). A1R-/- mice showed reduced exploratory behavior in the open-field and in the hole-board, increased anxiety in the dark/light box and increased offensive aggressivity in the resident-intrudent test. The three genotypes showed similar acquisition patterns in all the paradigms in the Morris water maze for spatial reference and working memories. Survival curves until 2,5 age-old are also presented, showing a significantly reduced survival rate in A1R-/- as compared to A1R+/+.
This work was supported by Swedish Medical Research Council K2000-04X-12587-03A and FIS 99/1230. L.G LL is recipient of a MEC reincorporation contract.

Human Behavioral Genetics: Making Connections
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Human Behavioral Genetics: Making Connections. The enormous wealth of information resulting from the human genome project's success has opened new frontiers of opportunities for deciphering the elusive molecular components of human behavior. As has been the case for diabetes, cardiovascular disease, hypertension, Alzheimer's disease and other complex trait medical disorders, we have gained a better appreciation of the diversity of possible susceptibility and protective factors involved in the multifactorial mode of inheritance of normal and abnormal human behaviors. The advantages of comparative studies of genomes and behaviors from a wide variety of model organisms are increasingly appreciated. These revolutionary changes in our approaches to identifying the molecular substrates of behavior, coupled with the availability of increasingly higher throughput instrumentation, data mining and analyses, have enabled new avenues for exploration of the variations in complex trait components that contribute to a wide range of behaviors. The emergence of new directions in our thinking about the molecular substrates of human behavior will certainly have a major impact on clinical diagnosis and treatment.

Fellow travellers on the road to the genetics of behavior: Mice, Rats and Dogs
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The Mendelian conception that, like the periodic table and Darwinian evolution, has enabled us to reach a new level of understanding of varied and complex natural phenomena based on a few seminal expeiments that lent thmselves to simple interpretations and opened new horizons. The neatness of the experiments that exactly conformed to his conceptual scheme has raised questions about the relations between the two. In addition to pea plants and honeybees, Mendel also bred mice of different coat colors and found that these segregated, a finding that very likely gave him the idea of independent assortment and of its general applicability. That mice showed physical and behavioral variations that could breed true, was known to the ancient world where these were prized,as in the case of waltzing mice, and mice have remained an important organism for behavioral genetic research to this day, when the possibility of matching mouse to human genes and transferring genes to study their contributions on different backgrounds and in different has become possible, permitting new understanding of how genes relate to behavioral capacities.

Plastic changes underlying behavioral sensitization to cocaine and morphine: A comparative behavioral and neurochemical study in the Roman high- (RHA) and low-avoidance (RLA) lines of rats

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Repeated administration of psychostimulants, like cocaine, or opiates, such as morphine, causes behavioral sensitization (BS), which is characterized by enhanced drug responsiveness with repeated doses. BS is associated with enduring plastic changes in the functional properties of a variety of neural systems, including the mesolimbic dopaminergic (DAergic) pathway. The selection of Roman high- (RHA) and low-avoidance (RLA) rats for rapid vs. poor acquisition of active avoidance in a shuttle-box has led to additional genetically determined differences. Thus, these lines differ in several neurochemical processes and behaviors modulated by the mesolimbic DAergic system, including the responses to acute administration of psychostimulants and morphine. The present study was aimed at assessing the influence of genetic factors on the development of BS by comparing: (1) the behavioral responses of RHA and RLA rats to acute and repeated cocaine or morphine, and (2) the effects of repeated morphine administration on DA function in the "shell" and "core" compartments of the Nucleus Accumbens (NAshell and NAcore, respectively) of these two lines. In the 1st experiment, RHA and RLA rats received daily injections of cocaine (10 mg/kg, i.p., 14 days) or an equivalent volume of vehicle (Veh). Motor activity in basal conditions and in response to acute cocaine (5 and 10 mg/kg) or Veh was assessed one day before (pre-test, PT) and 10 days after (challenge, CH) the repeated injections of cocaine or Veh. The results obtained indicate that : (1) in the PT, cocaine-stimulated (5 and 10 mg/kg) ambulatory activity was more intense in RHA rats relative to their RLA counterparts, and (2) repeated cocaine injections produced BS only in RHA rats. In the 2nd experiment, each line received twice daily injections of morphine for 3 days (5, 10, and 20 mg/kg, SC in the 1st, 2nd and 3rd days of treatment, respectively) or an equivalent volume of vehicle (Veh). Motor activity in basal conditions and in response to acute morphine (0.5 mg/kg, SC) or Veh was assessed one day before (e.g., PT) and 21 days after completion of the repeated treatment schedule (e.g., CH). As in the case of cocaine, ambulatory activity after acute morphine was more intense in RHA than in RLA rats, and repeated morphine injections produced BS only in RHA rats. In the 3rd experiment, brain dialysis probes were implanted in the NAshell and NAcore, 20 days after completion of the repeated treatment schedule with morphine or Veh and neurochemical assays were performed 24 h after surgery. No significant differences across line or treatment were observed in the basal DA output in either accumbal compartment. Moreover, in rats treated repeatedly with morphine, a subsequent challenge with the same drug produced a more robust increase in DA output (i.e., DA augmentation) in the NAcore of the RHA line, which developed BS, but not in the RLA line, which failed to do so. In contrast, the DA response to a challenge with morphine was blunted in the NAshell of sensitized RHA rats, but remained unchanged in non - sensitized RLA rats. The experimental protocols described herein may represent valuable models to examine the neurochemical mechanisms underlying the development of BS in comparative studies using genetically selected laboratory animals, like the Roman rat lines. Further studies are warranted to investigate the neural systems and processes involved in the opposite changes in DAergic transmission in different accumbal compartments that occur in association with BS. Such studies may contribute to our understanding of the neural basis of BS and drug dependence.
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Alcoholism candidate alleles and haplotypes in the Gaba/Serotonin domain

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Alcoholism is a complex disease involving pharmacogenetic variation in drug metabolism and response. Genetically influenced paharmacodynamic differences in alcohol response include

pre-morbid drug seeking behavior, acute responses to alcohol including sedation and reinforcement, and long-term tolerance and craving. In the human, alcohol response is predictive of future addiction and in animal models it is predictive of preference. GABA and serotonin are components of functionally interacting neurotransmitter systems that have been repeatedly implicated in the pharmacodynamic differences in ethanol response in rodent pharmacobehavioral and genetic models. Four GABAA receptor gene clusters are represented by mouse ethanol quantitative trait loci. Also, certain receptors for both GABA and serotonin are ethanol responsive. Both neurotransmitters are key, and functionally interact, in anxiety/dysphoria, which appears to predispose to drug self-administration and to be an important mediating phenotype in one subtype of alcoholism. 5HTTLPR, an abundant functional serotonin transporter promotor polymorphism [Lesch et al], appears to be a gene for anxiety/ dysphoria and has been implicated in alcoholism risk. The in vitro functional effect of this polymorphism, sib pair linkage to trait anxiety [Mazzanti et al], and in vivo functional data [Heinz et al] will be reviewed. Whole genome linkage scans in SW Indians [Long et al] and Finns [unpublished] implicate the Chr 4 and Chr 5 GABAA clusters in alcoholism. We have followed up these genome scans with linkage and haplotype linkage disequilibrium studies to the narrower GABAA cluster region on chromosome 5. Receptors of particular interest in this gene cluster are the g2 receptor, which is required for alcohol response, and the a6 receptor, for which an amino acid substitution was previously found to determine differences in alcohol and benzodiazepine response in rats. In SCID-interviewed Finns [110 alcohol dependent and 124 controls] and SADS-L interviewed SW Indians [192 alcohol dependent and 241 controls] linkage to a six-locus GABAA receptor haplotype was detected. TrimHap [MacLean et al, 2000] was used to estimate the most probable location of the functional locus within the Chr 5 cluster. In both populations that location was within the GABAA a6 receptor or in the interval between this receptor and the g2 receptor gene. We previously reported Pro385Ser, a nonconservative amino acid substitution of GABAA a6. The Ser385 allele has a allele frequency of 0.04 [Iwata et al]. In relatively small human datasets, this allele was associated with both benzodazepine response [Iwata et al] and alcohol response [Schuckit et al]. In the latter report, evidence was presented for an interaction between Pro385Ser and 5HTTLPR to determine alcohol response.

Gene Networks and the Subtleties of Behavior
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Naturally occurring variants in behavior have generally been difficult to analyze due to the multi-genic nature of the differences between strains and the relatively weak contribution of each locus, in contrast to the more drastic mutations conventionally isolated in behavioral screens and mapped by standard Mendelian genetics. Tropisms and taxes are characteristics that have long been studied by means of selection, and that exhibit complex, multi-genic inheritance. Understanding the molecular differences between such selected lines and manipulating variants of the individual genes provide important points of entry into the study of the gene networks underlying behavior and the genetic flexibility in an organism's response to its environment.

Genes and Behavior in the Fly: From Natural Variants to Induced Mutants and Back Again
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The application of genetic analysis to behavior in *Drosophila* has evolved in step with general advances in genetics. The power of genetic analysis in *Drosophila* has repeatedly been called upon to test behavior genetic issues, many of which have arisen elsewhere. A synthesis is now under way between the traditionally separate studies of natural variants and induced mutants.

Role of fosb in nicotine-induced tolerance and reward

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Our previous work demonstrated that the transcription factor FosB contributes to cocaine's abusive properties (Hiroi et al., 1997). We have now tested the hypothesis that FosB contributes to nicotine's abusive properties, using fosB knockout (KO) mice. First, we examined the development of tolerance to nicotine's motor depressant effect. Wild-type (WT) mice gradually developed tolerance to the motor depressant effect of nicotine (0.8 mg/kg, s.c.) over 2 weeks, but KO mice developed complete tolerance within 3 days. This finding suggests that FosB opposes the processes that underlie the development of tolerance. Second, we assessed the role of FosB in nicotine's rewarding and aversive effects in the place-conditioning paradigm. WT mice exhibited conditioned place preference at low doses (0.2 and 0.4 mg/kg, s.c.) and conditioned place aversion (CPA) at high doses (0.8 and 2 mg/kg, s.c.). By contrast, KO mice exhibited CPA at all doses tested (0.1, 0.2, 0.4, 0.6, 0.8, and 2.0 mg/kg, s.c.). This finding suggests that FosB plays a role in nicotine's rewarding, but not aversive effects. These two sets of experiments suggest that FosB plays complex roles in distinct aspects of nicotine addiction. This work was sponsored by a grant from the NIDA, R01DA13232-01 and by grants from the Program in Human Genetics/Howard Hughes Fund, AECOM to NH. Noboru Hiroi, Ph.D. Director, Laboratory of Molecular Psychobiology.

Genetic background determines behavioral phenotypes in serotonin transporter knockout mice

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The membrane bound serotonin transporter (5-HTT) is a key regulator of serotonergic neurotransmission, controlling the duration and intensity of serotonergic actions at the synapse. In order to study the role of the 5-HTT in emotional behavior and body weight regulation, mice with a targeted gene deletion of the 5-htt gene (5-HTT KO) mice were separately backcrossed onto C57BL/6J (B6) and 129SvEvTac (129S6) genetic backgrounds. 5-HTT KO on both backgrounds were normal on measures of general health, neurological reflexes, gross motor functions, and sensory functions, as compared to wild type littermates. 5-HTT KO on the B6 background showed an age-related obesity and performed poorly on the accelerating rotarod test for motor coordination. On the 129S6 background, 5-HTT KO were normal on these measures but showed impaired neuromuscular strength on the wire hang test. 5-HTT KO displayed profound increases in anxiety-related behaviors on 5 separate tests on the B6 background, while on the 129S6 background, the anxiety-related phenotype in 5-HTT KO was sex-dependent and confounded by general decreases in locomotor exploration. Regardless of background, 5-HTT KO mice showed enhanced emotional memory in cued and contextual fear conditioning. 5-HTT KO on the 129S6 background showed reduced immobility on the tail suspension test and increased immobility on the forced swim test, but normal scores on the B6 background. Genetic background has been shown to be an important influence on the behavioral phenotypes of knockout and transgenic mice. Present findings demonstrate that genetic background is a critical determinant of behavioral phenotypes in 5-HTT KO mice. Identifying background genes that modify the effects of major genetic perturbations, such as deletion of the 5-htt, could provide valuable insights into the genetics of complex traits. Supported by NIMH (IRP).

Behavioural search strategies of TgCRND8 mice during swimming navigation learning
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Transgenic (Tg) mice expressing a compound mutant form of human APP (TgCRND8) exhibit increased levels of A 42 in the brain, and show A plaque deposition and disrupted spatial navigation in the series of learning reversal reference memory Morris water maze tests (4 trials/day, different location of a hidden platform each day). In the test mice perform unconstrained swim search of the pool area to locate a submerged escape platform. Efficient acquisition of spatial information is usually inferred from short escape latencies to the platform, and behaviourally manifests itself through a direct swim path to (spatial strategy) or intensive search within close proximity of the escape platform (focal strategy). Other non-spatial strategies such as searching the pool at a constant distance from the wall (chaining response), scanning, or random search of the entire pool may account for the observed improvement in escape latencies. We extended the analysis of the swimming path data to evaluate search strategies used by the mice in order to elucidate cognitive and non-cognitive factors contributing to poor performance of transgenic mice in the test. The TgCRND8 mice showed a profound deficit in learning a hidden platform position during learning reversal tests. An analysis of the swim search strategies revealed that the transgenic mice searched for the platform primarily using a non-spatial strategy (chaining) or searched in the wrong quadrant of the pool. In contrast non-Tg mice used predominantly spatial search strategies.

Quantitative Trait Loci For Locomotor Activity in Drosophila
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Adult locomotor activity is a quantitative trait exhibiting continuous phenotypic variation in populations attributable to multiple quantitative trait loci (QTL) with effects that are conditional on genetic background and the environment. We developed a simple assay to quantify activity of Drosophila melanogaster, and demonstrated a highly significant difference in activity between two highly inbred strains, Oregon and 2b. Locomotor activity was assessed for each of 98 recombinant inbred lines derived from these strains. There was highly significant genetic variation among lines (P>0.0001) as well as sex-specific genetic variation (P=0.0008) for activity. Four main effect activity QTL were mapped by linkage to polymorphic roo transposable element insertion sites using composite interval mapping. These QTL include: one on the X chromosome that affects both sexes (1B-3E), one on the chromosome 2 affecting only females (27B-29E), one on chromosome 2 affecting both sexes (30D-38A), and one on chromosome 3 that affects female activity (98A-99A). A fifth QTL was discovered on the tip of chromosome 3 (99B-100A) that affects only male activity when epistatic tests were conducted. There were extensive epistatic interactions among these QTL. Results from quantitative deficiency complementation tests to fine-map the activity QTL and identify candidate genes will be presented.
This work was supported by an NIH pre-doctoral fellowship to KWJ and NIH grants GM 45344 and GM 45146 to TFCM.

The structure of individual differences in mouse (Mus musculus) intelligence: Modular or general processes?

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For the past several years our laboratory has studied the structure of individual differences in mouse problem-solving. The objective of this work is to examine whether general-process or modular models provide the more appropriate description of this structure. Our results suggest that as we have increasingly diversified our batteries in terms of task type and motivational system studied, the results have encouraged an increasingly modular model of individual differences. These results in turn question the appropriateness of general-factor models of mouse intelligence.

Individual Differences in Learning and Synaptic Function in CD-1 Mice

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We have developed a test battery designed to assess the general learning abilities of individual mice. The battery is comprised of six tasks that were included and designed so as to satisfy four requirements.
1) Each task makes unique demands on the animal, requiring diverse information processing strategies and impinging on distinct sensory, motor, and motivational systems.
2) An animal's experience with one task should not obviously influence its performance on other tasks in the battery.
3) To minimize differential influences of time and to accommodate practical considerations (e.g., the integration of animals into larger studies), tasks were designed such that each could be completed in one or two days, requiring 20 days to administer the complete battery.
4) Rate of acquisition served as the index of learning in each task, precluding any contaminating influence of differences between individuals in their capacity for long-term retention.
Moreover, the assessment of acquisition rate insures our sensitivity to real differences in learning, i.e., our measures are devoid of floor or ceiling affects on performance.In initial tests of 24 outbred CD1 mice, individuals exhibited distinct differences in their aggregate performance (e.g., average rank across tasks relative to peers), indicative of a general influence on learning that transcends task-specific idiosyncrasies. Moreover, animals aggregate learning abilities were strongly predicted by their tendency to engage in certain exploratory behaviors, suggesting that learning was regulated in common with the propensity of an animal to engage its environment.

The Genetic Architecture of Complex Behaviors in Drosophila

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Behaviors are typically quantitative traits, for which continuous variation in natural populations is attributable to multiple interacting quantitative trait loci (QTL), with effects that are contingent on the genetic background and the environment. Understanding the genetic basis of variation for complex behaviors in natural populations thus requires that we enumerate and localize QTL affecting variation in the traits; ascertain the QTL effects, singly and in combination; determine the effects on other quantitative traits; and define QTL alleles at the molecular level. This level of genetic dissection of quantitative traits is currently feasible only in genetically tractable and well characterized model systems, such as Drosophila melanogaster. The first step towards understanding the genetic architecture of quantitative traits is to map QTL associated with

variation in the trait, by linkage to phenotypically neutral molecular markers. However, QTL defined in this manner are chromosome regions containing many genes. Methods for higher resolution mapping and confirmation of the existence of the QTL in Drosophila include introgression of QTL alleles into a standard background, and deficiency complementation mapping. Even these methods will not typically resolve the QTL map positions to single loci, but will restrict the number of possible genetic loci corresponding to the QTL to 10-100 genes. Quantitative complementation tests can then be used to determine which of these genes interact with QTL alleles, if mutant alleles exist. Screens for viable P transposable element-induced mutations with quantitative phenotypic effects define novel pleiotropic effects of known loci and functions of predicted genes, and provide mutant stocks that can be used in quantitative complementation tests. Finally, linkage disequilibrium mapping is used to ascertain which molecular polymorphisms within candidate genes are associated with quantitative variation in phenotypes. The results of the application of these methods to determine the genetic basis of Drosophila olfactory behavior, mating behavior and adult locomotion will be presented.
This work was suppported by grants from the National Institute of Health and by the W. M. Keck Center for Behavioral Biology.

Behavioral and neuroanatomical characterization of FVB/N inbred mice
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The inbred strain FVB/N is becoming increasingly popular to generate transgenic animals. We compared animals from this strain to well-characterized C57BL/6J animals on four different behavioral tests: the elevated plus maze test of anxiety, a standard opponent aggression test, the open-field, and spatial learning in a radial maze. Our results indicate that FVB/N animals have slightly higher levels of anxiety and aggression, are hyperactive, and have a clear learning deficit. The latter finding seems to be related to an exceptionally small intra- and infrapyramidal mossy fiber projection. It is recommended that transgenic experiments employing this strain use F1 crosses between FVB/N and C57BL/6J as much as possible for behavioral experiments intended to evaluate spatial learning.
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Mutant Presenilins Disturb Neuronal Calcium Homeostasis and Increase Production of Amyloid Peptides in the Brain of Transgenic Mice
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Alzheimer's disease (AD) is a major social and medical problem in our aging Western societies. Despite considerable progress in genetics, cell-biology and model making the precise pathogenic mechanisms involved still remain elusive. Dominant early onset familial AD (EOFAD) is mainly caused by mutant presenilin1 (PS1) and presenilin2 (PS2). Presenilins contain 6 to 8 transmembrane domains and are predominantly confined to the endoplasmic reticulum (ER). Employing transgenic mouse models we have shown that mutant human PS1

causes an Alzheimer's related phenotype in the brain of transgenic mice in combination with mutant human amyloid precursor protein by means of increased production of amyloid peptides. (1) that aggravate plaques and cerebrovascular amyloid (2). This gain of function of mutant PS1 is approached here in three paradigms that relate to glutamate neurotransmission. Mutant but not wild type human PS1 lowered the excitotoxic threshold for kainic acid in vivo, (ii) facilitated hippocampal long-term potentiation in brain slices, and (iii) increased glutamate-induced intracellular calcium levels in isolated neurons. Prominent higher calcium responses were triggered by thapsigargin and bradykinin, indicating that mutant PS modulates the dynamic release and storage of calcium ions in the endoplasmatic reticulum. In reaction to glutamate, overfilled Ca2+ stores resulted in higher than normal cytosolic Ca2+ levels, explaining the facilitated long-term potentiation and enhanced excitotoxicity. The lowered excitotoxic threshold for kainic acid was also observed in mice transgenic for mutant human PS2[N141I] and was prevented by dantrolene, an inhibitor of Ca2+ release from the endoplasmic reticulum (3).It is conceivable that all of the effects on calcium homeostasis of mutant PS are indirect and are mediated by increased cellular levels of amyloid peptides. However Increased [Ca2+]i in cultured cells is reported to increase Ab production and increasing hyperphosphorylation.of protein tau, which would make the deregulation of intracellular calcium homeostasis a prime candidate for all AD cases, both familial and sporadic. Further investigation has to clarify the pathogenic correlation between the calcium homeostasis and the amyloid peptide.

Quantitative Trait Loci Affecting Mating Speed in Drosophila melanogaster

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Quantitative Trait Loci Affecting Mating Speed in Drosophila melanogaster. Although mating behavior has been studied extensively in Drosophila, locating genes directly responsible for behavior has been limited due to the complexity of behavior and the multiple factors involved in its production. To locate candidate genes for mating speed, a D. melanogaster line selected for reduced mating propensity (2b3) was crossed to a line not selected for reduced mating propensity (Ore-R). These lines were then backcrossed to 2b3 and subsequently inbred, creating 98 recombinant inbred (RI) lines that have been scored for courtship latency and copulation latency. The lines were genotyped for polymorphic insertion sites of roo transposable elements and composite interval mapping was used to identify QTL responsible for variation seen in mating behavior. Three regions containing QTL for mating speed were formally significant based on permutation tests: one on the second chromosome (57C-57F) and two on the third (72A-85F, 96F-99A). Two additional regions approached significance: one on the X chromosome (1A-3E, Likelihood Ratio=17.2) and one on the third (61A-65A, LR=17.3). Data will be presented on the deficiency and recombination mapping that is being utilized to map these QTL with higher resolution. Complementation tests to mutations at candidate genes will further resolve the QTL to the level of genetic locus. North Carolina State University.
This work was supported by the W. M. Keck Center for Behavioral Biology.

Prepulse Inhibition of acoustic startle in a neurodevelopmental animal model of schizophrenia

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Developing a test that models the syndrome of schizophrenia is difficult due to the variety of symptoms (positive, negative and cognitive) that are associated with this disorder. The reduction in acoustic startle response produced by an acoustic prepulse is diminished in

schizophrenic patients and can be reversed by some neuroleptics. We have shown effective disruption of PPI (startle:120dB;background noise: 65dB; prepulses: 68,72,76dB) in normal rats by both dopamine agonists and NMDA antagonists. Initial experiments have also shown reversal of apomorphine induced disruption of PPI by neuroleptics. There is evidence that neonatal lesions (ibotenic acid) of the ventral hippocampus in rats result in a range of abnormal behaviors in the adult animal, which are not observed until after puberty (Lipska et al, 1993), and that this may be considered as an animal model of schizophrenia. Animals with bilateral hippocampal lesions or sham injected controls, were tested for PPI before (PD35) and after (PD56) puberty. Results showed that at PD 35 there was no difference between the sham and lesioned animals, however significant PPI was observed in PD56 lesioned animals. Experiments are being done to see whether these PPI effects are exaggerated in the presence of apomorphine and whether these effects could be reversed by acute and chronic neuroleptic treatment.

DHEAS and inhibitor of the steroid sulfatase activity (COUMATE) induce anxiolytic-like effects in CBA/H mice

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DHEAS and inhibitor of the steroid sulfatase activity (COUMATE) induce anxiolytic-like effects in CBA/H mice.The steroid sulfatase (STS) enzyme regulates the formation of dehydroepiandrosterone (DHEA) from dehydroepiandrosterone-sulfate (DHEAS). DHEAS is a negative allosteric modulator of GABA A receptor-gated chloride channels. It is classified as an excitatory neurosteroid. Surprisingly DHEAS has been shown as exhibiting both anxiolytic- and anxiogenic- like activity in mice in the elevated plus maze. The present study examines behavioral effects of STS inhibition by COUMATE and DHEAS injections in CBA/H mice in the elevated plus maze. COUMATE at 10 mg/kg and DHEAS at 5 mg/kg caused anxiolytic response by increasing in the percentage of entries in open arms. The number of closed arms entries showed that COUMATE and DHEAS had no effect on the motor activity. Nevertheless the mechanisms implicating DHEAS and sulfated neurosteroids in anxiety are not very well understood. They do not involve probably GABA A receptor pathways. These effects could be mediated via sigma 1 receptors as indicated by recent experiments. Our results suggest that STS activity and neuroactive steroids including DHEAS may play a role in the control of stress response in rodents.

Neurobehavioral phenotyping of sensitivity to addictive drugs

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Mice of the inbred strain C57BL/6J and DBA/2J have been extensively characterized for their differential sensitivity to behavioral effects of drugs of abuse. The reported experiments investigated strain-dependent differences for mesocorticolimbic dopamine

High-throughput screening of ENU mutagenized mice in the conditioned fear paradigm

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In order to identify the set of genes involved in a well-characterized memory paradigm, we performed a high-throughput screen on a large group of N-ethyl-N-nitrosourea (ENU)

mutagenized mice. Male C57BL/6J mice (G3) were derived from backcrossing G2 females to mutagenized G1 males, which enabled the detection of both dominant and recessive mutations. Mice were tested in the conditioned fear paradigm to detect impairments in hippocampus-dependent (contextual) and hippocampus-independent (tone) fear memory. Both types of fear memory were assessed at 1 day and 3 weeks after learning. Several candidate mutants were identified, most of which had selective impairments in the learning or consolidation of contextual fear memory. Currently, candidate mutants are bred to wild-type females to determine the heritability of the phenotype. Candidate mutants are also bred to other mouse strains for mapping of the mutation. Several candidate mutants were detected in multiple litters of the same family, which indicates a heritable trait.

Brain imaging and behavioral genetics in worms
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The analysis of simple, genetically tractable organisms has provided critical insights into the nature of many complex biological processes, including gene regulation and mul-ticellular development. We are using a similar approach to understand the molecular and cellular basis of behavior in the nematode Caenorhabditis elegans. By combining genetic analysis with quantitative behavioral analysis and in vivo neural imaging, we ultimately hope to gain a reductionist understanding of how specific neurons and gene products influence a whole animal's behavior. We have applied optical imaging techniques to quantify the ef-fects of genes on specific behaviors in this organism, and to measure the activities of neu-rons and muscle cells in live animals. To characterize the mutant phenotypes of genes in-volved in nervous system function, we have used an automated tracking system to record the behavior of individual animals over long time periods. We have used this system to analyze the roles of specific neurons and neurotransmitters on particular features of egg-laying be-havior and locomotive behavior, and gained insight into how the neural circuitry of the worm couples these two behaviors.

To fully understand behavior at the molecular and cellular level, it is necessary to determine how specific gene products affect the activity of identified neurons, and to corre-late the activity of these neurons with behavior. Genetically-encoded optical sensors, such as the FRET-based, ratiometric calcium-sensitive protein cameleon, have many potential ad-vantages for cell-specific non-invasive neural imaging. The use of optical indicators is par-ticularly attractive in C. elegans due to the animal's transparency, the ease with which trans-genic animals can be generated, and the difficulty of electrophysiological methods. How-ever, because of their relatively slow kinetics and small signal size, it has been difficult to use genetically-encoded sensors like cameleon in excitable cells. We have recently over-come these hurdles and developed imaging methods that have allowed us to detect and measure in vivo calcium transients in the mechanosensory touch receptor neurons of C. ele-gans in response to sensory stimulation. Using this technique, we have begun to address the molecular basis for these neurons' response to touch stimuli. We have found that the N-type voltage-gated calcium channel UNC-2 is necessary for touch-activated calcium tran-sients as well as for behavioral responses to touch. In contrast, recordings of calcium tran-sients from mutants defective in the putative mechanotransduction channel MEC-4 showed occcasional calcium transients in response to touch, suggesting that the touch neurons may contain a mec-4-independent sensory modality, perhaps involved in harsh touch detection. Finally, we have observed that repeated high-frequency stimulation of the touch cells results in a reduction in the calcium response to successive stimuli; experiments to determine the mechanistic basis for these experience dependent changes in touch neuron function are in progress.

C. Elegans as a Prototype for Behavioral Analysis: Reductionistic, Modular, and Systems Biology Approaches

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Completion of the genomic sequence of C. elegans and other organisms has refocused attention and research programs on "functional genomics" and more "global" approaches to gene-organism relations. The most promising current and near-term strategy to achieving better understanding of multiple gene actions involves DNA microarrays. Very recent work using this technology applied to C. elegans has been published or is in press, including papers from Kim's group at Stanford and Chalfie's laboratory at Columbia. DNA microarrays that can track the simultaneous expression of many thousands of genes seem especially promising in the neuroscience area because of the complexity of the nervous system and the many-many relationship between neurons and genes. However, there are difficulties of both a technical and conceptual nature involving microarray investigations in neuroscience. This paper briefly reviews DNA microarray research in C. elegans, with some pointers to yeast, E. coli, mice, and human analyses, and develops an analytical framework in which interpretations of microarray results are situated. The framework examines the joint need for well-characterized reductionistic dynamical models, higher-level modular decompositions, and systems integration of data from genomics, proteomics, and enviromics. Elements from models for representing and handling biological complexity will be drawn from the work of Lander, Hartwell and Hopfield, Karp, and Hood's group, and will be explored for application to C. elegans. Several specific strategies for experiment interpretation including cluster analysis, Bayesian causal nets, and computational symbolic theory approaches are reviewed.
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Genetic Interactions Underlying Absence Seizure

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Complex interactions among multiple genetic components may contribute to the genesis of absence seizure, which is characterized by a brief loss of consciousness accompanied by an EEG recording of bilaterally synchronous spike-and-wave discharges (SWDs). We tried to define the role of calcium channels in the SWD seizures by using mutant mice for multiple isotypes of voltage-gated calcium channels. First, we observed that a1A(-/-), knock-out mice for the a1A subunit of high-voltage-activated (HVA) Ca2+ channel, showed 3-Hz SWDs associated with behavioral absence seizures. The cellular mechanisms underlying the SWD seizures were sought by electrophysiological examination of thalamic relay neurons. Whole-cell patch clamp analysis showed that low-voltage-activated (LVA) T-type calcium currents were increased whereas HVA currents were reduced in the a1A(-/-) neurons, suggesting a role for T-type Ca2+ channels in the genesis of the absence seizure in a1A(-/-). To examine this possibility further, we generated a knockout of the a1G subunit of T-type channels. The a1G(-/-) mice were resistant to the generation of SWDs in response to GABA-B receptor activation. To examine gene interactions between the two isotypes, the double mutants were obtained. EEG analysis showed that SWDs of a1A(-/-) disappeared in the double mutants, revealing a genetic suppression of the a1A(-/-) seizure by the a1G mutation. We conclude that an alteration of the profile of voltage-gated Ca2+ channel currents, LVA vs HVA, leads to the pathological synchronization in the thalamocortical network, resulting in absence seizures.

Transcriptome chromosome maps: A novel resource for complex trait analysis of the mouse CNS

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Rapid advances in RNA expression analysis and expanding bioinformatic resources now make it possible to study genome-wide variation in expression patterns in the CNS. Surprisingly, genome-wide differences in expression level have not yet been mapped back onto the genome to test whether there are systematic relations between chromosome location and expression level. We generated a database of just over 4000 mapped genes represented on the Affymetrix U74Av2 mouse chip. Mapped elements are a subset of a sample of 12480 genes. Arrays were probed in three regions—olfactory bulb, forebrain, and cerebellum. RNA was usually pooled from three isogenic males taken single litters and caged separately for ~ 1 wk. Background compensated and averaged data were log normalized. Database were assembled from the Portable Gene Dictionary, MGD, and NCBI resources. Statistical and exploratory analysis relied on DataDesk . We assayed relative expression levels in CNS compartments by principal components method. The major axis represent common expression. Regional differences were plotted in a projection plane orthogonal to the principal axis for each chromosome. These chromosome-brain transcriptosome maps provide an intuitive way to assess expression differences in a multidimensional space. Many of the usual gene suspects are highlighted by this analysis (e.g., Omp in bulb; En2, Pcp1, Cbln1 in cerebellum; Ache in forebrain). However, a large number of unexpected and novel genes have regionally intense expression and some of these may correspond to QTLs that we have mapped for bulb, cerebellum, and hippocampus.
We thank David R. Shaw of the Jackson Laboratory for computationally mapping Affymetrix probe sets and GenBank accession numbers. We thanks Dr. Divyen Patel and colleagues at GenomeExplorations (www.genomeexplorations.com) for excellent support in processing RNA samples and generating Affymetrix array data sets.

Underlying dimensions of aggressive behavior in male wild house mice

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The aim of this study was to get insight into the factorial structure of offensive aggression in male wild house mice. To this end, we assessed multiple measures of aggressive behavior in a design that focused on two key contexts for aggression: familiarity with the test cage and familiarity with the opponent. Thus genetically selected aggressive mice (SAL, characterized by Short Attack Latencies) and genetically selected non-aggressive mice (LAL, characterized by Long Attack Latencies) were tested in four paradigms:
[1] A reverse resident intruder paragigm, in which the opponent is the resident and the experimental animal the intruder.
[2] A resident intruder paragigm, in which the experimental animal is the resident and the opponent the intruder.
[3] A neutral cage paradigm, in which both animals are simultaneously placed in a test cage.
[4] A "naturalistic" paradigm, in which the alleged encounter takes place in a special cage, which mimics the situation in nature.
The experimental animals were tested twice in each test, against different standard opponent males (A/J and DBA/2), on consecutive days. In the naturalistic test the males were also tested against a female. A rotated principal component analysis reveals two factors: a general

aggression factor with particularly high loadings from the resident intruder paradigm and the naturalistic paradigm. It explains about 60% of the variation and seems to identify mainly territorial aggression. The second dimension, which explains about 10% of the variation, appears to reflect a more extreme, almost pathological type of aggressive behaviour. For instance, the inverse resident intruder paradigm loads highly on this factor, but only the test against the 'docile' (A/J) opponent and not the test against the more 'provocative' (DBA/2) opponent. The highest loading, however, comes from the test against a familiar female. These measures seem to tap in a more unnatural dimension of aggressive behaviour, which seems to have no social function.

Identification of neuronal nicotinic receptor gene family missense SNPs in mice

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Inbred mouse strains are a valuable resource for identifying genetic loci that contribute to individual variability in anatomical, behavioral, biochemical and physiological measures. Our lab is interested in understanding the influence of genetic heterogeneity among the neuronal nicotinic receptor subunit (nAChR) genes on development, disease, and drug abuse. In order to determine the potential utility of numerous inbred mouse strains for such studies, we have screened 32 inbred strains for missense SNPs in several nAChR subunit genes. Missense SNPs were identified in the Chrna4, Chrna6, Chrnb2 and Chrnb3 genes. Alleles defined by the missense SNPs for Chrna4, Chrna6 and Chrnb3 were distributed across common inbred strains and inbred strains derived from wild mice. In contrast, the missense SNPs for Chrnb2 were confined to inbred strains derived from wild mice. No missense SNPs have been identified to date for Chrna7 in either the common inbred strains or the Inbred strains derived from wild mice. A missense SNP in Chrna4 has been shown to be associated with mouse strain differences in nAChR function and preliminary electrophysiological data suggest that the SNP influences receptor function by altering the equilibrium between two affinity states of Chrna4/Chrnb2 receptors. The affect of Chrna6, Chrnb2 and Chrnb3 missense SNPs on receptor function have not yet been evaluated.
This work supported by the National Institutes of Health (DA-14369), The Alcoholic Beverage Medical Research Foundation, and The University of Michigan Tobacco Research Network.

Symposium: QTLs meet Mutagenesis

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Recent years have seen a dramatic increase in the search for genes underlying complex diseases. In the mouse, the methodologies being used have been focused on two primary efforts: random mutagenesis and quantitative trait loci (QTL) analysis. In this symposium we review these variant approaches and will discuss advantages and disadvantages in the analysis of brain and behavior. One of the key advantages of complex trait analysis is the ability to genetically dissect hard phenotypes such as those studied by many behavioral neuroscientists. A disadvantage is the difficulty of pin-pointing responsible gene variants. The conversion of QTLs to QT genes is now poised to enter a new phase. One advance relies on greatly expanded panels of recombinant inbred (R)I strains that include from 50 to 250 lines. The power of these RI sets can be amplified by making the large numbers of F1 intercrosses (RIX). RIX analysis makes it possible to explore epistasic interactions, gene pleiotropy, and reaction norms using non-inbred populations of isogenic mice, making them suitable for collaborative longterm

studies of comparatively "normal" mouse populations. The serious practical advantage of mutagenesis screens compared to QTL is that new phenotypes are much more readily tracked as single locus variables and candidate mutations are more readily indicted. However, one cannot be completely ignorant of natural variants in mutagenesis screens because, as will be discussed in detail using examples, they may be reintroduced in the outcrosses required to genetically map the new mutations. The idea of marked, regional mutagenesis of the mouse genome will be presented and discussed as well. A natural outgrowth of the regional approach is that one can use mutagenesis as a viable means to go from QTL to gene. Some efforts in this direction are proposed.

Genetic basis for differential geotactic behavior in selected lines of Drosophila melanogaster analyzed with DNA microarrays and mutants

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Now classical experiments by Jerry Hirsch and Theodosius Dobzhansky into the nature of the genetic basis for extreme, selected geotactic behavior in fruit flies constituted the first attempt at the genetic dissection of a complex, polygenic behavior. These studies demonstrated a genetic basis for geotaxis and by implication, behavior in general. Understanding the genetic differences between these selected lines would provide an important point of entry into the study of genetic mechanisms of sensing and responding to gravity, as well as clues to the origins of genetic flexibility and plasticity in an organism's response. To identify particular genes, we have screened the two extreme geotactic lines, Hi5 and Lo, using cDNA microarrays. Differences were found in a wide variety of genes. We obtained flies mutant for a set of these genes and ran them on a Hirschian geotaxis maze and found that some significantly changed the geotaxis behavior of the fly. These results report the demonstration of genes affecting a selected, polygenic behavior, provide insight into the nature of genetic change due to over 40 years of lab based selection and isolation, and report the first confirmation of cDNA microarray technology on a behavioral phenotype.

Assessing the reliability of tests of behavior in standard inbred mouse strains

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A good test of behavior should be highly reliable. Because theory derived from human psychological testing assumes things we know are not true of mice, we have devised methods more suitable to the dynamics of animal behavior. First, we assess the consistency of change within and across trials for an individual mouse using multiple regression. Large differences among strains are evident for these quantities. Then we consider reliability when data from all mice in the sample are combined. By using both approaches, we can show that a test is highly reliable, even if strains happen not to differ very much on a particular measure. The methods are applied to the study of activity (open field and Y maze), motor coordination (accelerating rotorod), and learning/memory (Morris maze) using 8 of the 9 standard mouse strains on Priorilty List A of the Mouse Phenome Project (www.jax.org/phenome) plus the B6D2F1 hybrid. Inbred strains include A/J, BALB/cByJ, BTBR/J, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ, and 129S1/SvImJ. The CAST/Ei strain proved to be too difficult to handle in some tests and was dropped. We use these methods to determine the minimum length of testing required to yield acceptably high reliability with high throughput and to select tests for use with genetic screening. The influence of a strain with extreme scores on the degree of reliability is also considered.
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Quantitative genetic analysis of diapause in Drosophila melanogaster

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We have undertaken a genetic analysis of diapause in Drosophila melanogaster. Diapause is a hormonally-mediated, delayed response to future adverse conditions (Danks, H. Insect Dormancy an Ecological Perspective) and can occur at any stage of development in an insect. In Drosophila melanogaster, it occurs as an adult ovarian diapause that is triggered by conditions of low temperature and short days (Saunders et al. 1989 PNAS USA 86:3748-3752). Specifically, flies in diapause have immature ovaries that remain previtellogenic. We placed flies that were less than 10 hours post-eclosion into 11 C and 10L:14D for 16 days after which we determined the percentage with previtellogenic> ovaries and called this the percent diapause. We used two appraches to find genes that affected diapause: a) Quantitative trait locus (QTL) analysis using recombinant inbred lines with roo transposable element markers, b) Candidate gene approach. We measured the diapause phenotype of 75 molecularly marked recombinant inbred lines. Cytogenetic region 97D-E on chromosome-3 provided the only significant QTL indicating thatr the genetic etiology behind the diapause phenotype in these lines could be relatively simple. We plan to further localize the gene or genes that affect diapause using deletions and quantitative complementation analysis. The age-1 gene affects a reproductive arrest in Caenorhabditis elegans called dauer formation. age-1, is homologous to Dp110 in Drosophila. Both encode a phosphoinositide-3 kinase (P13K) known to be expressed in the eye-antennal discs, wing imaginal discs and photoreceptors in Drosophila. We use the GAL4-UAS targeted expression system to over-express Dp110 in the nervous system and eye. When resticted to the nervous system (elav-GAL4), or almost exclusively to the R1-R6 photoreceptors (GMR-GAL4), the augmented expression of Dp110 promotes development of the ovaries under diapause-inducing condtions. Reducing the gene dosage of Dp110 using a deletion of the Dp110 genomic region increased the diapause response. Future studies will determine whether it is Dp110's function in the insulin signalling pathway that is responsible for the diapause phenotype. This work represents the first genetic analysis of the diapause phenotype; we have one gene(Dp110) and one QTL that affect diapause in Drosophila melanogaster.
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Integrated Complex Trait Analysis of Brain and Behavior in Mouse: Near-term Prospects for High-Resolution QTL Mapping using Large RI and RIX Sets

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The analysis of complex multigenic traits in mice is poised to enter a new phase in which QTLs can be routinely mapped to subcentimorgan intervals. This advance relies on the accumulation of defined recombination breakpoints in large panels of RI strains (>50 members). For example, the BXN multi-allele RI set consists of about 85 extant strains from Jackson Laboratory (www.nervenet.org/papers/bxn.html). The LXS diallele RI set from B Bennett and TE Johnson (IBG, U Colorado) consists of ~85 RI strains. The BXD set will soon comprise over 80 strains (L Lu, J Peirce, LM Silver, RW Williams). Novel advanced RI (ARI) lines made using 4- and 8-way

crosses (>250 lines), each of which will incorporate 100-160 breakpoints, will have the mapping utility of 3 conventional RI strains. The new RI intercross method (RIX mapping) significantly enhances the power of RI resources and makes it possible to explore epistasic interactions, gene pleiotropy, and reaction norms using completely non-inbred populations of isogenic mice. RIX lines are ideal for collaborative behavioral genetic studies. We will describe the genetic structure of RI, ARI, and RIX lines and illustrate how we are using an RIX cross to map CNS biometric traits (hippocampus, cerebellum, olfactory bulb, whole brain) with surprising power and precision.

Escape strategies of mice in the watermaze: a meta-analytical dissection

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Hippocampal lesions in rats selectively disrupt place navigation in the Morris swim navigation task. Based on this observation, variants of this test are widely used to assess spatial learning and memory in genetically modified mice. However, careful observation of performing mice reveals that multiple forms of learning and a variety of escape strategies are involved. Spatial navigation is only a final step in a complex learning process. Mutations affecting procedural components of learning, behavioral flexibility or motivation may interrupt the process at early stages, long before spatial navigation abilities become a limiting factor. We have used a standardized watermaze procedure for most of our strain comparisons and studies of genetically modified mice conducted during the past 15 years. In this procedure, mice are first trained for a fixed goal during three days and then have to learn a new position during the following two days. Using principal component analysis (PCA) and 120000 video-tracked swim paths from 4000 mice we have now evaluated a large set of parameters across different strains and mutations, and have grouped them according to their ability to quantify particular escape strategies. We find that escape performance during training is mainly determined by non cognitive factors. Measures of escape performance have little predictive value for the precision and intensity of searching during a later probe trial, which are commonly taken as measures of spatial memory. On the other hand, PCA suggests that a mere quantification of searching behavior during a probe trial may underestimate the degree of spatial learning in many mice. In conclusion, watermaze experiments provide more information about the behavioral abilities of mice than commonly assumed. However, careful analysis and combination with suitable control experiments are needed to avoid misinterpretations and fully exploit this information.

Initial behavioral characterization of galanin receptor GAL-R1 knockout mice

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Galanin is a neuropeptide that modulates neuroendocrine secretion, gastrointestinal motility, pain transmission, ingestive behavior, anxiety-like behavior, and cognition. Galanin signaling is mediated by three distinct receptor subtypes which have been designated GAL-R1, GAL-R2, and GAL-R3. In the present experiments, we describe the initial behavioral characterization of GAL-R1 knockout mice. The behavioral characterization employed a battery of tests assessing the general health, neurological reflexes, motor, sensory, and cognitive abilities of GAL-R1 -/-, +/-, and +/+ littermates of both genders at ages six to nine months. We found no effect of genotype on the general health and reflex function of the mice. There was also no effect of genotype on performance on open field locomotion, accelerating rotarod, acoustic startle, prepulse inhibition of acoustic startle, hot plate test, tail flick test, or olfactory tests. GAL-R1 knockout mice were impaired in a test of olfactory memory, the social transmission of food preference task.