

INTERNATIONAL BEHAVIOURAL AND NEURAL GENETICS SOCIETY

KNOCKOUTS & MUTANTS II: Genetically Dissecting Brain and Behavior
SECOND ANNUAL GENERAL MEETING

October 21 - October 22, 1999

Key Largo, Florida, USA

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to be announced

Local Host

Wim E. Crusio (Orléans, France)

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Program

Wednesday, October 20, 1999

6.00-8.00 pm Registration and Welcome Reception (Poolside)

Thursday, October 21, 1999

7.00 am-5.30 pm Registration

8.00-8.30 am *Opening session*

8.30-9.30 am NEUROGENETICS Lecture. S. Tonegawa (Cambridge, MA, USA). Studies on learning and memory, and activity-dependent development of the visual system with genetically engineered mice.

9.30-10.30 am *Contributed paper session. Phenotypical analysis of induced mutations. Chair: Douglas Wahlsten (Edmonton, Alberta, Canada)*

9.30-9.45 am D. Moechars, I. DeWachter, E. Godaux., B. Cordell. and F. Van Leuven (Beerse, Belgium). Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain.

9.45-10.00 am K.E. Browman, J.C. Crabbe and T.J. Phillips (Portland, OR, USA). Behavioral sensitization to the locomotor stimulant effects of ethanol and morphine in 5-HT1B knockout and wild-type mice.

10.00-10.15 am G.A. Carlson, S.K. Turner, D. Peterson, and J. Gilchrist (Great Falls, MT, USA). Application of chemical mutagenesis to dissecting neurodegenerative disease pathways.

10.15-10.30 am E.N. Pothos (New York, NY, USA). Regulation of monoamine quantal size by the neuronal vesicular transporter VMAT2.

10.30-11.00 am Coffee Break

11.00-12.30 am *Symposium. Suicidal behaviour and genetic polymorphisms of the 5HTT gene. Chair: Philip Gorwood (Colombes, France)*

11.00-11.30 am P. Gorwood, P. Batel, J. Ades, M. Hamon, and C. Boni (Colombes, France). Serotonin transporter gene polymorphisms, alcoholism and suicidal behaviour.

11.30-12.00 am E. Duaux, J.L. Laplanche, P. Gorwood, M.C. Bourdel, J.M. Launay, and M.F. Poirier (Paris, France). Role of the serotonin transporter promoter polymorphism in opiate dependence and suicide attempts.

12.00-12.30 am F. Bellivier, A Szöke, C. Henry, J. Lacoste, C. Bottos, M. Nosten-Bertrand, P. Hardy, R. Rouillon, J.M. Launay, J.L. Laplanche, and M. Leboyer (Creteil, France). Association between serotonin transporter gene polymorphisms and suicidal behavior in patients with affective disorders.

12.30-2.00 pm Lunch break

2.00-3.00 pm *Poster Session I*

1/ J.P. Allen, N.J. Clarke, S. Topps, M.I. Jowett, P.P.A. Humphrey, and P.C. Emson (Cambridge, UK). Analysis of somatostatin receptor 2 (*sstr2*) gene expression in *sstr2* knockout/*lacZ* knockin mice.

2/ V.J. Bolivar, D. Pierce, and A. Messer (Albany, NY, USA). The development of behavioral abnormalities in Huntington's disease (HD) transgenic mice.

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- 3/ B.J. Caldarone and M.R. Picciotto (New Haven, CT, USA). Role of high affinity nicotinic receptors in learned helplessness behavior.
- 4/ S. Chiavegatto, T.M. Dawson, V.L. Dawson and R.J. Nelson (Baltimore, MD, USA). Serotonin is involved in the aggressive behavior of male nNOs null mice.
- 5/ F. Cirulli, C. Rondinini, A. Venerosi, G. Calamandrei, and E. Alleva (Rome, Italy). Prenatal administration of 3'-azido-3'-deoxythymidine (AZT) affects adult intermale aggressive behaviour in mice.
- 6/ M.N. Cook, E.A. Vonnegut, V.J. Bolivar, and L. Flaherty (Albany, NY, USA). Are knockout and transgenic mice really telling us what we want to know about behavior?
- 7/ M. Dierssen, X. Altafaj, J. Guimerà, X. Estivill, and C. Fillat (Barcelona, Spain). Transgenic mice overexpressing the rat *minibrain* gene (*Dyrk1a*): implications for Down syndrome.
- 8/ C.L. Dockstader, M. Rubinstein, D.K. Grandy, M.J. Low, and D. van der Kooy (Toronto, Ontario, Canada). The D2 receptor, but not the D1 receptor, is critical in mediating opiate motivation when mice are opiate-dependent and in withdrawal.
- 9/ J.C. Fentress (Dalhousie, Nova Scotia, Canada). Tracing behavioral phenotypes in neurological mutant mice.
- 10/ R. A. Hensbroek, A. Kamal, M. Verhage, and B.M. Spruijt (Utrecht, The Netherlands). Impaired synaptic function results in hyperactive behaviour but not cognitive dysfunction in gene dose mutant mice for the neuronal protein munc18-1.
- 11/ Holmes, J.G. Hohmann, R.A. Steiner, and J.N. Crawley (Bethesda, MD, USA). Behavioral phenotype of transgenic mice with overexpression of the neuropeptide galanin.
- 12/ Janus, A.M. Chishti, D. Westaway, and P. St. George-Hyslop (Toronto, Ontario, Canada). Familial Alzheimer disease mutations in the presenilin 1 gene disrupt the rate of acquisition of spatial information.
- 13/ M.A. Joiner and L.C. Griffith (Iowa City, IA, USA). Anatomical localization of the CaM kinase II requirement for learning and memory in the courtship conditioning assay in *Drosophila*.
- 14/ Sarah L King, M.B. Kelz, C. Steffen, J. Chen, E.J. Nestler and M.R. Picciotto (New Haven, CT, USA). Trigenic mice with spatially and temporally restricted expression of high affinity nicotinic acetylcholine receptors.
- 15/ Benoît Martin, Patricia Zerr, and John P. Adelman (Orléans, France). The murine *Bis1* seizure gene and the *Kcnab2* gene encoding the $\beta 2$ -subunit of K^+ channel are different.

3.00-4.00 pm Plenary lecture. R. Rose (Bloomington, IN, USA). Behavior-genetics of use and abuse of alcohol.

4.00-4.30 pm Coffee Break

4.30-6.00 pm Symposium. Schizophrenia: Flies, Mice, and Man. Chair: Wim E. Crusio (Orléans, France)

4.30-5.00 pm M. Karayiorgou (New York, NY, USA). COMT- and PRODH-deficient mice as models for genes predisposing to psychiatric disorders.

5.00-5.30 pm S. Leonard, K. Stevens, P. Bickford, L. Adler, R. Freedman (Denver, CO, USA). Use of rodent models of an auditory gating deficit for identification of candidate genes in schizophrenia.

5.30-6.00 pm R. Paylor (Houston, TX, USA). Mouse genetic models for sensorimotor gating deficits.

6.00-7.00 pm IBANGS Business Meeting (Members only)

7.30-10.00 pm Caribbean Buffet (Beach)

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Friday, October 22, 1999

8.00 am-5.30 pm Registration

8.00-9.00 Plenary Lecture. T. Tully (Cold Spring Harbor, NY, USA). Genetic basis of memory.

9.00-10.00 am Contributed paper session. Learning and Memory. Chair: Leonard Meltzer (Ann Arbor, MI, USA)

9.00-9.15 am H.-P. Lipp, W.E. Crusio, and D.P. Wolfer (Zürich, Switzerland). Simple experimental solutions to the genetic background and flanking gene problems.

9.15-9.30 am R. Gerlai, D. Choi-Lundberg, L. Powell-Braxton, and H.S. Phillips (South San Francisco, CA, USA). GDNF heterozygous mutant mice in two genetic backgrounds exhibit spatial task specific cognitive deficits in the water maze.

9.30-9.45 am C.E.E.M. Van der Zee, C. Jost, F. Oerlemans, M. Verheij, B. Wieringa, and A. Cools (Nijmegen, The Netherlands). Impaired learning behavior and hyperactivity observed in Brain Creatine Kinase (BCK)-deficient mice is emphasized in double mutants (BCK/UbCK-deficient) and in aged BCK-deficient mice.

9.45-10.00 am S. Baron and L. Meltzer (Ann Arbor, MI, USA). Response acquisition: a rapid comparison of learning between mouse strains.

10.00-10.30 am Coffee Break

10.30-12.00 am Round Table "Solutions for the genetic background problem in KO and transgenic mice". Chair: Hans-Peter Lipp (Zurich, Switzerland).

Participants: W.E. Crusio (Orléans, France), R.T. Gerlai (South San Francisco, CA, USA), and D.P. Wolfer (Zurich, Switzerland).

12.00-12.30 Presentation by Noldus Information Technology b.v

12.30-2.00 pm Lunch break

2.00-3.00 pm Poster Session II

- 16/ Tsuyoshi Miyakawa and Jacqueline N. Crawley (Bethesda, MD, USA). Image analysis software for behavioral phenotyping of mutant mice.
- 17/ J.J. Pancrazio, G.M. Grant, C.L. Wilson, D.A. Stenger, J.D. Andreadis, G.D. Ritchie, M.Y.V. Bekkedal, and J. Rossi III (Wright-Patterson AFB, OH, USA). Gene expression variability in cortical neurons: implications for genomic in vitro prediction of neurobehavioral compromise.
- 18/ F. Petty, G.L. Kramer, and M.L. Kram (Dallas, TX, USA). Learned helplessness and dopamine receptors.
- 19/ I.I. Poletaeva, L. De Acetis, L. Ricceri, M.G. Pleskacheva, N. Markina, A.L. Vyssotski, D.P. Wolfer, E. Alleva, and H.-P Lipp (Moscow, Russia). Intermale aggression and hippocampal mossy fibers in naturally selected mice.
- 20/ I.Y. Ponomarev and J.C. Crabbe (Portland, OR, USA). Genetic associations between chronic ethanol withdrawal severity and acoustic startle parameters in WSP and WSR mice.
- 21/ F.O. Risinger (Portland, OR, USA). Quantitative Trait Loci for acute behavioral sensitivity to Paraoxon.
- 22/ L Rondi-Reig, M Libbey, H Eichenbaum, and S Tonegawa (Cambridge, MA, USA). CA1 LTP: a synaptic mechanism of memory flexibility?

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- 23/ A.G. Sadile (Naples, Italy). The phenotypic expression of the behavioral trait of the Naples High Excitability rat-line is modified by environmental factors during early post natal life.
- 24/ M. Sedelis, K. Hofele, I. Willuhn, S. Morgan, G.W. Auburger, J.P. Huston, R.K.W. Schwarting (Düsseldorf, Germany). Strain differences in the MPTP mouse model of Parkinson's disease: In search of the underlying genetic mechanisms.
- 25/ A. Shamir, G. Sjholt, R. Lovlie, R.H. Belmaker, R.P. Ebstein, G. Agam, and V. Steen (Beer Sheva, Israel). Characterization of mouse inositol monophosphatase genes.
- 26/ A. Smith, M. Keneshige, S.-Y. Cheng, and M.P. McDonald (Nashville, TN, USA). Phenotypic analysis of mice bearing a mutant human thyroid beta receptor.
- 27/ B.J. Snyder, S.P. Baron, and L.T. Meltzer (Ann Arbor, MI, USA). Developmental changes in the behavioral phenotype of different mouse strains.
- 28/ L. Stanford and R.E. Brown (Halifax, Nova Scotia, USA). Multivariate analyses of anxiety in inbred mice using the open field, novel object recognition and elevated plus maze.
- 29/ R.F.G. Toonen, J.P.H. Burbach, and M. Verhage (Utrecht, The Netherlands). In vivo analysis of Munc18-1 protein interactions. Transgenic approach on a Munc18-1 deficient background.
- 30/ Weller, A. Leguisamo, L. Towns, and D. Brunner (Hawthorne, NY, USA). "Maternal environment" affects the offspring's behavior, in 5-HT-1A and -1B -receptor knockout mice.
- 31/ V. Zachariou, A. Weathers-Lowin, B. Caldarone, T. George, J.P. Changeux, and M.R. Picciotto (New Haven, CT, USA). Knock out of the high affinity receptor for nicotine decreases sensitivity to cocaine.
- 32/ L. Ricceri, G. Dell'Omo, D.P. Wolfer, I.I. Poletaeva, and H.P. Lipp. Natural learning in mice: temporal and spatial adaptation of foraging behavior in a radial-maze analogue task under naturalistic conditions.
- 33/ F. Sluyter, M. Hof, T. Breivik, and A.R. Cools. Genetic and early postnatal effects on the sensitivity to periodontitis in Wistar rats.

3.00-4.00 Presidential Address. H.-P. Lipp (Zurich, Switzerland). Genes, Brain and Behavior: Bottom-up and Top-down Approaches.

4.00-4.30 pm Coffee Break

4.30-6.00 pm Contributed paper session. Social Behavior and Miscellaneous. Chair: Enrico Alleva (Rome, Italy)

4.30-4.45 pm B. Olivier, J.A. Bouwknecht and R. Hen (Hawthorne, NY, USA). 5-HT1b-knockout mice are impulsive: telemetric evidence.

4.45-5.00 pm E. Alleva, F. Cirulli, M. Bianchi, G.P. Bondiolotti, F. Chiarotti, L. De Acetis, and A.E. Panerai (Rome, Italy). Aggressive behaviour of interleukin-6 overexpressing or deficient mice.

5.00-5.15 pm S. Mortaud, L. Nicolas, and P.L. Roubertoux (Orléans, France). Attack behavior in mice: implication of the *sts* gene mapped on the pairing region of the X-Y chromosomes.

5.15-5.30 pm C. Chabert and P.L. Roubertoux (Orléans, France). Maternal behavior is impaired in mice lacking *Nos1*.

5.30-5.45 pm D. Wahlsten (Edmonton, Alberta, Canada). Proof of a third source of individual differences in brain structure that is neither hereditary nor environmental.

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5.45-6.00 pm O. Giorgi, D. Lecca, G. Piras, J.H. Medina, and M.G. Corda (Cagliari, Italy). Effects of stressors and antidepressants on central serotonergic transmission: A comparative behavioral and brain dialysis study in Roman High- (RHA/Verh) and Low-Avoidance (RLA/Verh) rats.

J.P. Allen¹, N.J. Clarke², S. Topps², M.I. Jowett², P.P.A. Humphrey³, and P.C. Emsen¹. Analysis of somatostatin receptor 2 (*sstr2*) gene expression in *sstr2* knockout/*lacZ* knockin mice⁴.

Functional roles for somatostatin have been implicated in many physiological processes, both in the central nervous system (CNS) and periphery. These actions are mediated through a family of five seven transmembrane receptors. A paucity of pharmacological compounds and the lack of animal models hinder in vivo analysis of somatostatin function. Therefore, through gene targeting technology, we have generated a somatostatin receptor 2 (*Sstr2*) knockout mouse. Concomitantly we have 'knocked in' the *lacZ* reporter gene into the *Sstr2* locus such that *lacZ* expression is controlled by the entire *Sstr2* regulatory machinery.

Preliminary observations in homozygous mutants reveal no overt phenotype, either morphologically or behaviorally. Further, heterozygous and homozygous intercrossing produces mice with genotypes at the normal Mendelian ratio.

lacZ expression analysis shows a distinct distribution in the CNS. Staining is seen in many areas including the neocortex, the hippocampus, the amygdala, the subiculum, the habenula, the claustrum, the striatum, the locus coeruleus, the hypothalamus and the central grey. In the spinal cord expression is present in the dorsal column and all laminae of the grey matter, but predominantly in laminae I and II (substantia gelatinosa). This pattern is largely in agreement with the receptor distribution as determined in the rat by somatostatin binding, in situ, and immunohistochemical studies, indicating that *lacZ* expression indeed recapitulates that of *Sstr2*. We therefore conclude that this animal model will provide a valuable tool for the detailed analysis of *Sstr2* expression. Further, the ability to easily identify *Sstr2* positive cells, via *lacZ* staining, will facilitate expression and functional studies at the cellular level.

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Enrico Alleva¹, Francesca Cirulli¹, Mauro Bianchi², Gian Pietro Bondiolotti², Flavia Chiarotti¹, Luigi De Acetis¹, and Alberto E.

Panerai². Aggressive behaviour of interleukin-6 overexpressing or deficient mice.

We investigated aggressive and affiliative behaviour exhibited during agonistic encounters by transgenic male mice either not expressing (IL-6 ^{-/-}) or overexpressing (NSE-hIL-6) interleukin-6 (IL-6) in the central nervous system. All subjects were isolated for 24 days before the aggressive encounter and were 52 days old at the time of testing. Subjects were placed for 5 consecutive days in a neutral cage for 15 min with an opponent of the Balb/c strain that had been previously isolated for the same amount of time. The 1st and the last test sessions were videorecorded to evaluate the first approach and the establishment of the social role respectively. A number of behavioural categories were later scored. When compared to their controls, IL-6 ^{-/-} mice showed a higher degree of aggressive behaviour as indicated by a higher frequency of Offensive Upright Posture, an effect more pronounced on the fifth encounter. On the contrary, NSE-hIL-6 subjects showed a tendency to be more involved in affiliative-type social interactions, displaying a higher frequency and duration of behaviours such as Anogenital, Nose or Body Sniff. IL-6 ^{-/-} mice showed a clear tendency to exhibit less affiliative interactions compared to their controls while dopamine levels were found to be modified in a number of brain regions in these mice. Overall, these data suggest that IL-6 exerts complex effects on both aggressive and affiliative-type behaviour.

¹Section of Behavioural Pathophysiology, Lab. F.O.S., Istituto Superiore di Sanità, Rome, Italy, ²Dept. of Pharmacology, University of Milan, Italy.

S. Baron¹ and L. Meltzer¹. Response acquisition: a rapid comparison of learning between mouse strains².

Characterization of learning phenotypes in different mouse strains has risen to prominence especially as it relates to genetic bases of behavior. A problem with many learning paradigms is the great length of training time required. Our goal for the current studies was to develop a method of rapidly assessing learning and validating the procedure using various strains of male mice. Prior to response acquisition food-deprived mice were acclimated for two hours to operant chambers and liquid dipper presentation of evaporated milk.

The following day mice were placed in operant chambers with two nose-poke holes illuminated. A single nose poke in one hole resulted in presentation of 0.01 ml evaporated milk for 10 seconds. Nose pokes in the other

hole resulted only in an audible click. Sessions ended after 120 dipper presentations or two hours. On day three of training the consequences of nose pokes were reversed. There were no non-contingent dipper presentations during acquisition or reversal training sessions. An acquisition criteria of 50 correct (reinforced) responses was set. The F1 generation hybrid of B6x129/SvImJ mice emitted 50 correct responses in 23 min in comparison to C3H/HeJ mice which, on average, emitted 50 correct responses in 50 min. C57Bl/6J and 129/SvImJ reached criteria after 34 and 48.8 min, respectively. Other strains such as BALB/cByJ and the outbred CD-1 mice performed at intermediate levels. In addition, rates of error commission decreased over the duration of the session. Subject emitted 50 responses more quickly under reversal conditions than under initial response acquisition. However, reversal learning did not maintain the same strain rank-order of performance. The findings with response acquisition are consistent with those reported using the same mouse strains in other learning paradigms and provide a rapid method of operant-learning assessment.

¹Parke-Davis Pharmaceutical Research, Plymouth Rd. 2800, Ann Arbor, MI 48105, USA.

²Supported by Warner-Lambert.

F. Bellivier¹, A Szöke, C. Henry, J. Lacoste, C. Bottos, M. Nosten-Bertrand, P. Hardy, R. Rouillon, J.M. Launay, J.L. Laplanche, and M. Leboyer. Association between serotonin transporter gene polymorphisms and suicidal behavior in patients with affective disorders.

Background: Genes implicated in the serotonin system are major candidates in association studies of suicidal behavior. In this case-control study, we investigated whether the serotonin transporter (5HTT) gene encoding the protein responsible for the re-uptake of serotonin from the synapse after its release from serotonergic neurons, is a susceptibility factor for suicidal behavior. *Methods:* Two polymorphisms of the 5HTT gene (a VNTR of the second intron and a 44 bp insertion/deletion in the 5HTT linked polymorphic region (SLC6A4**C*)) were studied in a population of 237 consecutive patients with affective disorder (unipolar or bipolar affective disorder) and 187 controls. Ninety-nine patients had attempted suicide at least once. *Results:* We found an association between the SLC6A4**C* polymorphism and violent suicidal behavior. For the SLC6A4**C* polymorphism, there was no difference between patients who had not attempted suicide and controls. For the VNTR

of intron 2, no difference was observed between patients with or without suicidal behavior and controls. *Conclusion:* A genetic variant of the 5HTT gene may predispose individuals to violent suicidal behavior. The precise phenotype associated with the 5HTT gene is unclear and therefore further studies are required to replicate these findings.

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Valerie J. Bolivar¹, David Pierce, and Anne Messer. The development of behavioral abnormalities in Huntington's disease (HD) transgenic mice².

Huntington's Disease (HD) is a progressive neurodegenerative disorder caused by a CAG/polyglutamine repeat expansion. Recent identification of the HD gene has led to the development of several transgenic models of this disorder. A mouse transgenic for exon 1 of an abnormal human HD gene (Mangiarini et al., 1996, *Cell*, 87,493) is currently the subject of many studies at cellular, biochemical and neuroanatomical levels. We are currently examining the behavior of these transgenic mice, with a focus on one particular late onset line (R6/1), which displays obvious neurological symptoms by 15-21 weeks of age. As the three main diagnostic criteria of Huntington's disease are motor abnormalities, cognitive impairment and emotional disturbance, we are currently examining behaviors relating to these three areas. We are using a battery of behavioral assays including exploratory activity in the open field, grooming in a small container, and fear conditioning to investigate the behavior of these mice. Our open field assay consists of 5-minute exposures to a dimly lit open field over three consecutive days. Grooming behavior is videotaped for 10 minutes before and after the mouse's fur is lightly sprayed with water. The videotapes are then analyzed frame-by-frame. Our fear-conditioning assay consists of three pairings of mild foot shock with a tone on the conditioning day and then subsequent measurement of contextual and cued memory 24 hours later. We are in the process of testing R6/1 mice from 4-20 weeks of age (16 transgenics and 16 littermate controls for each age group) with all of these behavioral assays. To date, we have found behavioral abnormalities as early as 8 weeks of age. As it appears that there are behavioral abnormalities weeks in advance of the more obvious neurological symptoms, we suggest that there may also be subtle early neuropathology.

¹Wadsworth Center, New York State Department of Health, David Axelrod Institute, Al-

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bany, NY, 12201-2002, USA. ²Supported by the Hereditary Disease Foundation.

K.E. Browman¹, J.C. Crabbe and T.J. Phillips. Behavioral sensitization to the locomotor stimulant effects of ethanol and morphine in 5-HT1B knockout and wild-type mice.

Repeated administration of addictive drugs commonly results in an increase in the locomotor stimulant response induced by acute administration. Termed behavioral sensitization, it is hypothesized that this augmentation may reflect a change in the reinforcing effects of addictive drugs. Recent evidence suggests that the serotonin 1B (5-HT1B) receptor subtype may modulate some drug-related behaviors, including ethanol (EtOH) and cocaine self-administration. We were therefore interested in investigating differences between 5-HT1B knockout and wild-type mice in sensitization to the stimulant effects of EtOH and morphine. In Exp. 1, male and female knockout and wild-type mice received daily injections of either 2.5 g/kg EtOH or saline for a total of 10 injections. Following chronic administration both saline and EtOH pretreated animals were challenged with 2.0 g/kg EtOH (a dose combination previously found to produce robust EtOH sensitization). In Exp. 2, mice of both genotypes were administered 15 mg/kg morphine or saline every other day for 10 days and challenged with 15 mg/kg. The genotypes exhibited similar susceptibility to EtOH sensitization. Conversely, knockout mice sensitized to the locomotor stimulant effects of morphine whereas wild-type mice did not. Our data suggest that the 5-HT1B receptor does not play a role in sensitization to the stimulant effects of EtOH, but may influence sensitization to morphine. These results suggest that the sensitized responses to EtOH and morphine are mediated by at least partially different mechanism(s).

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Barbara J. Caldarone¹ and Marina R. Picciotto. Role of high affinity nicotinic receptors in learned helplessness behavior.

Many studies have reported associations between smoking and depression. For example, depressed patients are more likely to smoke and less likely to quit smoking compared to the general population. These findings suggest that nicotine may act on nicotinic acetylcholine receptors (nAChRs) to alleviate the symptoms of depression. We have been investigating how nicotine may influence be-

havior in mice in the learned helplessness model of depression. In this model, animals administered inescapable foot shock show increased latencies to escape an escapable shock. In the present study we characterized learned helplessness in an outbred stock of mice and in the C57BL/6J (B6), 129/J (129) and B6129F1 strains, which are background strains for most knockout mouse models. Mice were administered either 60-6s, 120-4s, or 360-2s foot shocks and tested for shuttle escape behavior 24 hr later. Strain and sex differences were observed in learned helplessness. Outbred males exhibited the most robust helplessness response, B6 males and females showed an intermediate response, and outbred females, 129 and B6129F1 males and females showed no evidence of learned helplessness. Corticosterone levels were compared for B6, 129 and B6129F1 mice that received 360 shocks and their non-shocked controls. In general, 129 and B6129F1 mice had higher levels of corticosterone than B6 mice and females had higher corticosterone levels than males, but corticosterone levels did not vary with shock treatment. We are currently examining the role of the Beta-2 subunit of the nAChR in learned helplessness by comparing the response of knockout mice lacking the Beta-2 subunit of the nAChR with wild type mice, and have observed differences in the behavior of mutants and wild types in this paradigm.

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George A. Carlson¹, Sherry K. Turner, Dionne Peterson, and Julie Gilchrist. Application of chemical mutagenesis to dissecting neurodegenerative disease pathways².

The goal of our mutagenesis program is to identify genes and pathways with relevance to two neurodegenerative diseases, prion disorders and Alzheimer's disease (AD). Our first approach is to identify mutations in genes that are relevant to the functions of amyloid precursor protein (APP) or prion protein (PrP). The working hypothesis is that App or Prnp null-mutant mice will show phenotypes different from those seen in mice expressing these proteins when mutations occur in genes relevant to their as-yet-unknown functions. N-ethyl-N-nitrosourea (ENU) is the mutagen of choice for our program because it primarily induces point mutations that can produce either gain of function or loss of function. Both a first generation dominant screen and a three generation recessive screen are underway.

The screen, which takes approximately 5 minutes per mouse, targets behavioral or neurological abnormalities. Most of the mutations identified in the screen will not be in pathways relevant to App or Prnp function and many will be of little interest to scientists at McLaughlin Research Institute. Therefore, a list of phenodeviants and mutants is posted on the Internet and these mice will be available to the research community.

These studies will determine the feasibility of modifier screens in mice and compare alternative strategies for screening and mapping. Two distinct inbred strains carrying the each null allele will allow us to determine whether recovery of modifier mutations is more efficient when screening is done on an inbred background (with the risk of losing the phenotype when outcrossing for mapping) compared to screening and outcrossing simultaneously. To date (July 1, 1999) we have screened more than 1200 F1 mice and recovered 15 mutations, with more phenodeviants being progeny tested. In these initial studies, more than 30% of tested phenodeviants have been mutants.

¹McLaughlin Research Institute, 1520 23rd Street South, Great Falls, MT 59405, USA.

²Supported by an Alzheimer's Disease Research Grant from the American Health Assistance Foundation.

C. Chabert¹ and P.L. Roubertoux^{1,2}. Maternal behavior is impaired in mice lacking *Nos1*³.

A small number of genes correlated with maternal behavior have been reported yet, in contrast to those implicated in aggression or other social behaviors. We used mice in which *Nos1* had been invalidated. NOS was transferred after 20 backcrosses onto C57BL/6J background. Maternal behavior was investigated in primiparous female mice (80-90 days of age) using the retrieving test developed by Carlier *et al.* (*Behav. Neural Biol.* 35: 205-210, 1982). Data were collected within the 600 sec following the first contact of the mother with the pups. We measured 1) latency of retrievals, 2) duration between retrieval and placing the pup in the nest, 3) the time spent in the nest after placing of the first pup in the nest, 4) the number of times the female drew away from one of her pups, situated outside the nest, without their being transported, 5) time in the nest with all pups, and 6) the weight and size of the nest. Four groups of mice have been observed: *Nos* ^{-/-}, *Nos* ^{-/+}, *Nos* ^{+/+}, and C57BL/6J. No difference appeared between *Nos* ^{+/+} and C57BL/6J for the 6 variables. Nest size and

weight were similar in the four groups. Latency of first retrieving and duration between retrieval and placing the pup in the nest were longer in *Nos* ^{-/+} compared to *Nos* ^{+/+} and C57BL/6J. Latency of retrievals, duration between retrieval and placing the pup in the nest, the time spent in the nest after placing of the first pup in the nest, the number of times the female drew away from one of her pups, situated outside the nest, without their being transported, and time in the nest with all pups were different in *Nos* ^{-/-}, compared to controls and to *Nos* ^{-/+}. Implication of anxiety and olfaction in the observed impairment of maternal behavior were investigated.

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S. Chiavegatto¹, T.M. Dawson, V.L. Dawson and R.J. Nelson. Serotonin is involved in the aggressive behavior of male nNOS null mice².

We previously demonstrated that male mice with targeted disruption of the neuronal isoform of nitric oxide synthase (nNOS) display a marked increase in aggressive behavior with no neuroanatomical or physiological disturbances. This phenotype can also be induced with the selective nNOS inhibitor 7-NI in wild-type (WT) mice. Blood plasma testosterone levels do not differ between WT and nNOS^{-/-} male mice. Several lines of research have established that reduced brain serotonin (5-HT) levels result in increased aggression in a variety of species. To investigate the neurochemical profile of these mice, adult male nNOS^{-/-} and WT were decapitated and the monoamines and their metabolites were determined by HPLC with electrochemical detection in the hypothalamus, cerebellum, mid-brain, hippocampus, amygdala, cerebral cortex, accumbens and striatum. Reduced serotonin turnover (5HIAA/5-HT) was observed in the hypothalamus, cerebellum and cortex, while a decrease in dopamine (DA) turnover (DOPAC/DA) was found in striatum in nNOS^{-/-} mice. In order to study the involvement of 5-HT in the behavioral phenotype, the resident-intruder test was assessed after >60% inhibition of 5-HT and 5HIAA levels using p-chlorophenylalanine (pCPA) or 300% increase with 5-hydroxytryptophan (5-HTP), the 5-HT precursor, in both WT and nNOS^{-/-} mice. The aggressive behavior of WT mice

with pCPA was increased to the same magnitude as the nNOS^{-/-} animals while the precursor of 5-HT completely abolished this behavior in both genotypes. These data suggest that reduced activation of the serotonergic system might be responsible for the aggressive phenotype of nNOS^{-/-} mice.

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F. Cirulli¹, C. Rondinini¹, A. Venerosi², G. Calamandrei², and E. Alleva¹. Prenatal administration of 3'-azido-3'-deoxythymidine (AZT) affects adult intermale aggressive behaviour in mice.

AZT is commonly administered in pregnant women and supposed to reduce risks of mother/newborn transmission of HIV. Medium and long-term effects of AZT on neurobehavioural development and possible long term effects are still poorly described, even though some modest alterations have been reported in animal models. Aim of the present study was to evaluate the long-term effects of prenatal AZT treatment on aggressive behaviour of adult male mice. Pregnant CD-1 mice were administered saline vehicle, 0.4 or 0.8 mg/ml AZT in drinking water from gestational day 10 up to the delivery. Social-aggressive types of interactions were assessed in their male offspring (10 mice in each treatment group) following a 4-week isolation period. Two types of encounters were used: a) a single 20-min encounter with an isolated same-strain opponent on postnatal day (PND) 90, or (b) five repeated pairings with a group-caged opponent (8-males groups) on PND 150. Dose-dependent changes of both aggressive and defensive components of the male specific agonistic pattern were evident only in the former test, AZT mice showing 'dominant-type' features more often than controls.

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Melloni N. Cook¹, Emiko A. Vonnegut, Valerie J. Bolivar, and Lorraine Flaherty. Are knockout and transgenic mice really telling us what we want to know about behavior?

As more genes become identified as "players" in complex behavioral traits, the use of knockout and transgenic animals can tell us

more about the specific contribution(s) of gene(s) to behavior. In the accompanying table, we have summarized a large portion of the literature reporting behavioral traits of knockout and transgenic mice. We have noted that factors such as the age and sex of the animals are important in determining phenotypic traits. More importantly, the genetic background of the animals seems to play an essential role. The majority of knockout and transgenic mice are produced on an inbred 129 or mixed 129,C57BL/6 background. Before we can attribute a phenotypic trait to a transgene, the phenotypic characteristics of the background strain should be taken into consideration. We have begun characterizing C57/BL6J (B6) and 129S3/ImVJ (129) mice for several behavioral tasks measuring memory, anxiety and activity. While both strains show evidence of habituation to the open field, 129 mice are less active than B6; they also make fewer total arm entries in the plus maze and fewer beam breaks in the zero maze. In the open-field activity test, 129 mice appear to be more anxious than B6, spending more time near the margins of the apparatus. In contrast, they are less anxious in the plus maze than B6, spending significantly more time in the open arms. Preliminary data suggest that the 129 and B6 mice do not differ in the time spent in open quadrants of the zero maze or in the latency to enter an open quadrant. The data from novel object exploration show that B6 mice enter the area containing the novel object more frequently than 129. Preliminary data also show that these strains differ for several neurochemical measures including monoamine levels. Future studies will also characterize behaviors of several 129 sub-strains.

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M. Dierssen¹, X Altafaj, J. Guimerà, X. Estivill, and C. Fillat. Transgenic mice over-expressing the rat *minibrain* gene (*Dyrk1a*): implications for Down syndrome².

The human *MNB* gene is located on human chromosome 21 in the Down syndrome critical region. Mutations in the *mnb* gene from *Drosophila* result in specific defect in neurogenesis and perturbed visual, olfactory and motor behavior as well as cognitive defect in odor-discrimination learning. Its mammalian homologue in rat, *Dyrk1A*, encodes for a dual specificity tyrosine/serine-threonine kinase. mRNA of *Dyrk1A* has been found to be ubiquitously expressed. It is widely expressed in brain, including the regions af-

ected in Down syndrome. Consistent with the importance of minibrain in cognition, YAC transgenic mice that overexpress *MNB* have specific learning and locomotor deficits in the Morris water maze.

To address the specific role of minibrain in brain and neurobehavioral development, and memory formation, we have generated transgenic mice overexpressing the *Dyrk1A* gene, under the control of the inducible methallothionein (MT) promoter. The MT promoter can direct expression of heterologous genes to a variety of fetal and adult tissues in transgenic mice. The *sMT/Dyrk1A* chimeric gene was microinjected into fertilized mouse (C57B6/SJL) eggs and four lines of transgenic mice carrying different copy number of the transgene have been obtained. The transgene exhibits expression in all the tissues analyzed. To address the role of minibrain overexpression in mammalian development, somatometric, neurobehavioral and neuromotor development was studied in transgenic and control littermates from two founder lines. For the developmental studies, pregnant females were exposed to zinc ($ZnSO_4$ 25mM) treatment to further enhance the expression of the transgene during the embryonic and fetal period. No difference were found in the reproductive parameters analyzed, including number of pregnancies per matings, number of living pups and weight of pups at birth. Somatic and sensorimotor development of transgenic and wild-type mice from related litters was assessed from postnatal days 1 to 23. Pups were weighed daily, and measures of somatic growth were registered. Developmental landmarks in the preweaning period included pinna detachment, eyelid and ear opening, and incisor eruption. To evaluate sensorimotor development a wide range of reflexes as well as sensorial tests were assessed, that included surface righting reflex, negative geotaxis, and cliff aversion. Transgenic mice showed a retardation of walking activity and prolonged latencies in the homing test with respect to controls, that might be related to hypoactivity. Implications for Down syndrome phenotype will be discussed.

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**C.L. Dockstader¹, M. Rubinstein², D.K. Grandy², M.J. Low³, and D. van der Kooy¹.
The D2 receptor, but not the D1 receptor, is**

critical in mediating opiate motivation when mice are opiate-dependent and in withdrawal⁴.

According to the dual systems model for opiate reward, dopamine mediates opiate motivation when an animal is in a deprived motivational state (ie- opiate-dependent and in withdrawal) and not when the animal is in a non-deprived state (ie- previously drug-naïve). Congenic (backcrossed to the C57BL/6 strain five times; N5) D2 receptor-deficient mice (-/-) and their wild-type siblings (+/+) were run in a non-deprived, morphine conditioned place preference (CPP) paradigm. These previously drug-naïve mice demonstrated significant preferences for the morphine-paired environment regardless of genotype or morphine dose. In a deprived state, D2(+/+) mice acquired conditioned place aversions for a naloxone-paired environment as well as normal CPP for a morphine-paired environment whereas opiate-dependent and withdrawn D2(-/-) mice displayed a complete block in the acquisition of conditioned place aversion and preference. Similar paradigms were run with F₂ (not backcrossed and on a mixed 129/C57 background) D1 receptor-deficient mice and their wild-type siblings. While previously drug-naïve D1(-/-) mice demonstrated morphine CPP as well as conditioned place aversion when conditioned in a deprived state, the D1(+/+) mice showed no acquisition of either task. The unexpected phenotype of the D1(+/+) mice may be due to random fixation of detrimental 129 alleles (theoretically it could have occurred just as easily in the D1(-/-) line of mice). Mice derived from separate isogenic C57BL/6 and 129/SVJ strains were also tested for non-deprived morphine CPP. Although the C57BL/6 strain acquired a preference for the morphine-paired environment, the 129/SVJ strain showed no preference. We conclude that D2 receptor function is critical in mediating the motivational effects of opiates *only* when the animal is in a deprived motivational state whereas D1 receptor function is not critical (regardless of motivational state). Furthermore, these findings illustrate the important contributions that background strains can make to a given phenotype.

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E. Duaux¹, J.L. Laplanche, P. Gorwood, M.C. Bourdel, J.M. Launay, and M.F. Poirier. Role of the serotonin transporter pro-

motor polymorphism in opiate dependence and suicide attempts.

Background: Dysfunction of serotonin transmission could predispose to addiction behavior, and to aggressive or impulsive behaviors such as suicide attempts. The functional polymorphism in the human serotonin transporter (SLC6A4) promoter was identified and found to be linked with different disorders including severe alcoholism. *Methods:* We analysed the role of this polymorphism in a population of male opiate-dependent subjects. Fifty four male opiate-dependent-patients (DSM-III-R criteria), french for at least two generations, excluding schizophrenia, were personally interviewed with the DIGS (Diagnostic Interview for Genetic Studies) and the Barrat's impulsiveness scale, and compared to 63 unaffected blood platelet donors. *Results:* No association was found with opiate dependence, comorbid depression and score of impulsivity. An excess of homozygotes was found in patients without suicide attempts. The « S » allele was associated with suicide attempts (62% in controls, 65% in patients without suicide attempts, 85% in patients with suicide attempts, 100% in patients with 5 or more suicide attempts). *Conclusion:* Deficiency in 5 HT reuptake mediated by the short allele of the serotonin transporter gene seems to increase the risk for impulsive behaviors such as suicide attempts in our sample of opiate-dependent-patients.

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John C. Fentress^{1,2}. Tracing behavioral phenotypes in neurological mutant mice.

The full value of neurological, knockout, and transgenic mice for behavioral neuroscience obviously depends upon the establishment of measures for early detection and quantification of mutation effects. We have established behavioral tests that allow us to trace various patterns of sensory-motor coordination in inbred and neurological mutant mice (Weaver, Staggerer, Jimpy). We use development as a natural dissection tool to examine kinematic and sequencing properties of movement organization, and their relation to environmental perturbations. Recently we have focused upon the contributions of cerebellar and striatal circuits in the activation and patterning of movement under specific situations. Our data are obtained through video and related computer analyses of swimming, grooming, and exploratory actions. Movements are examined in terms of their kinematic details, limb trajectories and velocities, coordination among limb segments, higher-

order sequences, and in response to changing environmental demands.

Swimming allows us to examine early stages of movement coordination without the confound of gravity (and thus muscular strength plus balance problems). In myelin deficient jimpy mice developmental transitions in swimming are delayed, and phase relations between limbs are altered.

Grooming is a more complex and hierarchically ordered sequence of actions. Staggerer mice have cerebellar disorders that primarily affect movement form without disrupting sequences of actions, whereas Weaver mice also have dopamine deficiencies that are reflected in both movement activation and sequencing. Independent surgical manipulations suggest that these mutant mouse strains can be used to separate the operation of movement control mechanisms during maturation, with the added advantage that degeneration of operations in mutant animals can be compared with the progressive formation of movement in control ontogeny. Changes in responsiveness to external manipulations during different phases of ongoing movement clarify routes of sensory-motor integration.

An important goal in our current research is to explore the use of knockout and transgenic models that may further clarify the development and expression of sensory-motor patterns at different levels of organization. Our data suggest that combining developmental analyses of mutant mice with independent experimental manipulations during specific phases of ontogeny can provide converging data upon mechanisms of sensory-motor control at complementary levels of expression. However, progress will depend critically upon precise assessment of specific movement parameters and responses to sensory events as these unfold in ontogeny. I hope to discuss the potential value and also limitations of KO and transgenic mouse models in the design of further studies of behavioral ontogeny and its CNS substrates.

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R. Gerlai¹, D. Choi-Lundberg, L. Powell-Braxton, and H.S. Phillips. GDNF heterozygous mutant mice in two genetic backgrounds exhibit spatial task specific cognitive deficits in the water maze.

Glial cell line-derived neurotrophic factor (GDNF) is a potent neurotrophic factor for dopaminergic neurons in the mammalian brain. Research has been focused on brain areas (e.g. substantia nigra, striatum, and nucleus accumbens) where dopaminergic neu-

rons play crucial role and perhaps represent therapeutic targets for alleviating Parkinsonian symptoms. Recent studies, however, also showed that GDNF and their receptors, GFRalpha1, and c-Ret (the signaling receptor) are all expressed in the mammalian hippocampus, a brain area playing a central role in learning and memory. Furthermore, electric or ischemic stimulation induced transcription level changes in GDNF and its receptors have been reported in the hippocampus, and anecdotal evidence exists on systemic GDNF replacement therapy in humans leading to cognitive disturbances. Here we analyze heterozygous mice with a null mutation in GDNF. Pathologic and hematologic analyses showed that mutant mice do not suffer from gross abnormalities. Neurochemical analysis and tests of amphetamine induced locomotory behavior suggested that mutant mice possess an intact dopaminergic system. Interestingly, however, mutant mice exhibited a significant impairment in the spatial version of the Morris water maze, a task sensitive to hippocampal dysfunction. General performance factors, e.g. swimming speed or non-spatial learning, were unaltered. The results were replicated in two genetic backgrounds, a 129SV x C57BL/6 F2 hybrid and a C57BL/6 backcross (ninth generation) suggesting that the alteration is caused by the null allele. These results demonstrate that GDNF plays an important role in cognition associated with hippocampal function. They, together with previously published results, also raise the possibility that GDNF and its receptors may represent an important therapeutic target in epilepsy, ischemic brain insult, or neurodegeneration in the adult mammalian hippocampus.

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O. Giorgi¹, D. Lecca¹, G. Piras¹, J.H. Medina², and M.G. Corda¹. Effects of stressors and antidepressants on central serotonergic transmission: A comparative behavioral and brain dialysis study in Roman High- (RHA/Verh) and Low-Avoidance (RLA/Verh) rats.

The cortical projections of the serotonergic (5-HTergic) system are known to play a role in the acquisition of behaviors motivated by aversive stimuli. More recently, it has been shown that conditioned fear stress (CFS), an animal model of anxiety without physical stimuli, selectively increases 5-HT metabolism in the medial prefrontal cortex and produces marked freezing behavior, which is regarded as a reliable index of fear in rodents. The acti-

vation of 5-HTergic neurotransmission produced by some antidepressant drugs, like the selective serotonin reuptake inhibitors (SSRIs), is associated with a reduction in the frequency of defensive freezing in the CFS paradigm. These findings are in line with the view that the facilitation of central 5-HTergic neurotransmission has anxiolytic effects and make it possible to interpret the CFS-induced increase in cortical 5-HTergic function as a biochemical correlate of a cognitive function, such as "coping" with stress, rather than an emotional, "anxiety-related", reaction to aversive stimulation.

The Swiss sublines of Roman high-avoidance (RHA/Verh) and low-avoidance (RLA/Verh) rats are selected and bred for respectively rapid versus poor acquisition of two-way active avoidant behavior in a shuttle box. They have been shown to differ in several other behavioral aspects, most of which point to differences in emotionality and reactivity to stress, the RLA/Verh line being more reactive. Thus, when exposed to various unconditioned stressors, RLA/Verh rats show more pronounced tachycardia, a more intense activation of the hypothalamus-pituitary-adrenal (HPA) axis, and more robust anxiety-related behaviors, like freezing, than do RHA/Verh rats. A number of additional differences in dopaminergic, GABAergic, and 5-HTergic function in the CNS have been reported in these two lines. The behavioral and neurochemical characteristics of RLA/Verh rats suggest that this particular line could be used as a genetic model for studying the biological bases of anxiety and/or depression. The present study was therefore undertaken to examine line-related differences in 5-HTergic neurotransmission in the frontoparietal cortex (FPCx). To this aim, we compared the effects of tail-pinch stress (TP, 40 min) and anxiogenic doses of pentylenetetrazol (PTZ, 10 mg/kg, IP) on 5-HT output in the FPCx of the two lines, using brain microdialysis. We also compared the *in vivo* effects of 5-HT reuptake inhibitors administered systemically or intracerebrally on 5-HT output in the FPCx of RHA/Verh and RLA/Verh rats. In addition, the regional distribution of the 5-HT transporter was examined in the two lines by measuring the binding of the selective ligand [³H]-citalopram to coronal brain slices. The major findings obtained were as follows: (1) No statistically significant differences between RHA/Verh and RLA/Verh rats were found in the *basal* output of 5-HT in the FPCx. (2) TP and PTZ (10 mg/kg, IP) produced a more robust increase in 5-HT output in the FPCx of RHA/Verh rats than in their RLA/Verh coun-

terparts (maximal effect: ~ + 40 – 70% and + 5 - 20%, respectively). Aversive stimuli produced more frequent freezing episodes in RLA/Verh rats than in RHA/Verh rats. In contrast, RHA/Verh rats were much more active and persistent than RLA/Verh rats in their attempts to remove the clamp used to apply a mild pressure on their tails. This dissociation between behavioral responses and 5-HTergic activation across the lines is consistent with the view that the increment in cortical 5-HT output may reflect the activation of arousal- and/or cognition-related mechanisms in an attempt to cope with the stressor rather than one of the multiple neurochemical and hormonal adaptive reactions to the aversive stimuli. The systemic administration of chlorimipramine (CL, 10 mg/kg, IP) and fluoxetine (FL, 10 mg/kg, SC) produced a more robust increase in 5-HT output in the FPCx of RHA/Verh rats than in their RLA/Verh counterparts (maximal effect: ~ + 150 - 170% and + 70 - 80%, respectively). Likewise, the intracerebral perfusion of FL (100 μ M) through the dialysis probe caused a more pronounced increase in 5-HT output in the FPCx of RHA/Verh than RLA/Verh rats (maximal effect: 10-fold and 7-fold increment, respectively). In both lines, the systemic administration of CL or FL produced a significantly less pronounced increase in 5-HT output than the intracortical perfusion with FL. This difference is most probably due to the decrease in the firing rate of 5-HTergic neurons induced by the systemic administration of SSRIs. Such inhibitory effect results from the increment in the extracellular concentrations of 5-HT in the brainstem (which does not occur after intracortical perfusion with FL) and is mediated via the activation of 5-HT_{1A} autoreceptors located on the cell bodies of 5-HTergic raphè neurons. The density of [³H]-citalopram binding sites was significantly larger in the FPCx and other forebrain areas of RHA/Verh rats as compared to their RLA/Verh counterparts. The results of the brain dialysis and [³H]-citalopram binding studies support the view that the cortical 5-HT reuptake mechanism is more efficient in RHA/Verh than in RLA/Verh rats. Because the basal extracellular concentrations of 5-HT in the FPCx are not significantly different in the two lines, it appears reasonable to speculate that the cortical 5-HTergic tone must be more robust in the line in which the 5-HT reuptake mechanism is more efficient, that is, in RHA/Verh rats. Such hypothesis is in keeping with previous neurochemical studies and provides a neural substrate to account, at least in part, for the differences in the behavioral responses to aversive stimuli displayed by RHA/Verh and RLA/Verh rats.

Several neurotransmitters and hormones, including 5-HT, dopamine, norepinephrine, GABA and corticosteroids, are known to play a role in the reactivity to stress, as well as in the expression of emotion-related behaviors and the pathogenesis of mood disorders. Given the differences in neurotransmitter and HPA axis functions between RHA/Verh and RLA/Verh rats reported herein, these lines can provide two well-defined phenotypes to investigate the role of each of the above mentioned factors in the regulation of stress responses and in the biological mechanisms underlying anxiety and mood disorders.

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P. Gorwood¹, P. Batel, J. Ades, M. Hamon, and C. Boni. Serotonin transporter gene polymorphisms, alcoholism and suicidal behaviour.

Background: Dysfunction of serotonergic transmission could predispose to excessive alcohol consumption and dependence. The functional polymorphism of the serotonin transporter gene (5-HTTLPR) was actually associated with different disorders including alcoholism. Considering the likelihood of heterogeneity in the “alcohol-dependence” phenotype, the 5-HTTLPR may be more specifically implicated in sub-samples of patients, or in related traits of alcoholism, such as impulsivity. *Methods:* We analysed the role of this functional polymorphism in the risk for suicide attempt in a population of male alcohol-dependent-subjects. A hundred and ten male alcohol-dependent patients (DSM-III-R criteria), French for at least two generations, were personally interviewed with the DIGS, and compared to 61 unaffected blood-donors. *Results:* The “S” allele of the 5-HTTLPR appeared to be unrelated to alcohol-dependence and comorbid depression in our sample, but was found associated with an increased risk for suicide attempts. This association was predominantly observed in severe and repetitive suicide attempts, with a significant dose-effect of the “S” allele (0,1 or 2) on the number and the severity of suicide attempts. *Conclusion:* Mood disorders and alcohol-dependence may interact with a genetic (relative) deficiency in 5-HT reuptake, thereby increasing the risk for aggressive/impulsive behaviours such as suicide attempts.

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R. A. Hensbroek¹, A. Kamal, M. Verhage, and B.M. Spruijt. Impaired synaptic function results in hyperactive behaviour but not cognitive dysfunction in gene dose mutant mice for the neuronal protein munc18-1.

The neuron-specific protein munc18-1 is essential for secretion from presynaptic nerve terminals. Null mutants for munc18-1 have no secretion of neurotransmitters throughout the brain and consequently die at birth. Gene dose mutants (heterozygotes) have a normal reproduction and life span. Still, they do have a 50% reduction of munc18-1 expression. We hypothesize that a reduction in munc18-1 results in synaptic impairments and, as a consequence, in changes at the network level and at the behavioural level. In the hippocampus, depression during tetanic (5-20Hz) stimulation is more pronounced in the mutants. In addition, long term potentiation (LTP) in the CA1 region is reduced. This reduction is most pronounced in the first minutes after LTP induction. Most strikingly, mossy fibre LTP in the CA3 region is virtually absent. This form of LTP is NMDA-receptor independent and its induction and maintenance is reported to be mainly presynaptic. In their homecage, heterozygote mice were more active than wildtype mice. In addition, they also displayed a profound increase in locomotory behaviour in the open field test. We detected no differences in a fear test i.e. the light/dark box, suggesting that these changes in activity were not related to fear. The heterozygotes also showed impaired performance in cognitive tasks. In the eight-arm radial maze, a spatial learning task, their ability to collect food rewards was decreased. In the Morris water maze, however, they performed normal. In contrast to the dry land situation in the radial maze, swimming speed in the water maze was not increased. These results indicate that the increases in activity may have interfered with radial maze performance but not with Morris water maze learning. We conclude that munc18-1 reduction leads to impaired synaptic function as measured in the hippocampal network. Surprisingly, spontaneous behaviour seems more susceptible to these changes than cognitive function.

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A. Holmes¹, J.G. Hohmann², R.A. Steiner², and J.N. Crawley¹. Behavioral phenotype of transgenic mice with overexpression of the neuropeptide galanin³.

To explore the functional significance of the neuropeptide galanin in the nervous system, we generated transgenic mice overexpressing the galanin gene linked to a dopamine beta-hydroxylase promoter. Galanin mRNA in the locus coeruleus is approximately seven-fold normal levels, and galanin peptide is elevated in the forebrain. In order to study the role of endogenous galanin in behavioral processes we conducted behavioral phenotyping of galanin transgenic mice and their wild type littermate controls. Homozygous galanin overexpressing mice are viable, show no developmental abnormalities, and reproduce normally. Comprehensive evaluation of physical characteristics and neurological reflexes revealed that transgenic mice had no gross physical abnormalities, demonstrated normal eye blink, whisker twitch, visual cliff, and righting reflexes, and showed normal body weights and home cage behaviors. The motor performance of transgenic mice on the accelerating rotarod and Digiscan open field was similar to that of wildtype littermates. Analgesia tests indicated normal latencies in the hotplate and tailflick procedures. Given evidence that galanin may contribute to the etiology of mood disorders, transgenic mice were exposed to the elevated plus-maze and light/dark exploration tests for anxiety-like behaviors. As galanin is overexpressed in Alzheimer's disease, and galanin treatment produces performance deficits on learning and memory tasks in rats, galanin transgenic mice were tested in the Morris water maze, the Barnes maze, and cued and contextual fear conditioning. Initial findings indicate significant deficits on memory tasks, and are discussed with reference to cognitive disorders related to Alzheimer's disease.

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Christopher Janus¹, Azhar M. Chishti, David Westaway, and Peter St. George-Hyslop. Familial Alzheimer disease mutations in the presenilin 1 gene disrupt the rate of acquisition of spatial information.

Dominant mutations in the Presenilin 1 gene cause an aggressive, early-onset form of familial Alzheimer's Disease (FAD). To identify the role of such mutations in cognitive deficits in FAD, we examined transgenic mice expressing similar levels of either mutant or wild type (wt) human PS1 transgenes. Since it is accepted that the hippocampal region is af-

ected in the early stages of AD, we tested mice of these mutant (Tg(L286V)1274) and wild type (Tg(PS1wt)1098) lines at age 15 months in the cued and the place discrimination learning tasks using the Morris water maze (WM). All spatial learning tests were preceded by intensive non-spatial habituation to WM test conditions. We report that in the cue learning task, experimentally naïve Tg(L286V)1274 mice showed significantly longer swim paths than the Tg(PS1wt)1098 mice in the first part (5/11 days of training) of training, in the absence of differences in swimming abilities. Through manipulation of extra-maze training cues, we demonstrated that unlike the Tg(PS1wt) 1098 mice, the mutated Tg(L286V)1274 mice were unable to use more complex spatial strategies to locate the visible platform when a simpler, based on single cue association strategy was available. When the same groups of mice were trained longitudinally in conventional place learning task (4 trials/day, 10 days, the platform in the same spatial position), no differences in the acquisition of spatial information between the mutated and the wt mice were observed. However, in cross-sectional experiment using groups of experimentally naïve mice, mutated Tg(L286V)1274 showed an initial impairment in learning spatial information (first 5 days of 10-day training). Tg(L286V) 1274 mice were also significantly inferior in a place learning-set task (Whishaw, 1985. *Physiology & Behavior*, 35, 139-143) when a new place response had to be learned each day of training. We conclude that mice expressing mutant PS1 transgenes were: first, were unable to use more complex spatial strategies when a simpler one was available, and second were slower in the initial acquisition of new spatial information. The relationship of these findings to neuropathological changes in presenilin 1 Tg mice are discussed.

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M.A. Joiner¹ and L.C. Griffith². Anatomical localization of the CaM kinase II requirement for learning and memory in the courtship conditioning assay in *Drosophila*.

Global inhibition calcium/calmodulin-dependent kinase II (CaMKII) in whole animals leads to defects in both learning and memory. In the associative learning assay courtship conditioning, male flies learn to suppress courtship of virgin females by prior conditioning with a mated female. Females stimulate male courtship by both visual and positive

pheromonal cues. Mated females also give off an aversive pheromone, which acts as a negative cue. The amount of aversive pheromone which the mated female gives off increases as she is courted by the male. It is the association of these two pheromonal cues, with their different temporal profiles, that leads to suppression of courtship of the mated female and the subsequent inactivity toward the test virgin female.

To identify structures of the fly brain that require CaMKII for normal learning and memory, we express a CaMKII inhibitor under control of UAS, a yeast transcription factor (GAL4) binding sequence. Males with a GAL4 insert expressing the inhibitor peptide throughout the brain show both learning and memory defects. Localization and level of expression of GAL4 is assayed by β -galactosidase activity in GAL4;UAS-lacZ males and by confocal imaging of GFP in GAL4;UAS-GFP males.

Using a series of enhancer trap lines expressing GAL4 in limited regions of the CNS, we have assayed the performance of flies expressing the inhibitory peptide in each of these areas of the brain in GAL4;UAS-inhibitor males. GAL4 lines that express at high level in the mushroom bodies or parts of the central complex show memory deficits when expressing the inhibitory peptide under UAS control. Defects in the response to the mated female during conditioning are revealed in lines expressing the inhibitor peptide in the antennal lobe. Expressing the inhibitor peptide in other brain regions or in the thoracic ganglion does not appear to have an effect on learning or memory. Memory can be rescued in males that globally express the inhibitor peptide by co-expressing a wild-type isoform of CaMKII.

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M. Karayiorgou¹. COMT- and PRODH-deficient mice as models for genes predisposing to psychiatric disorders.

Previous work from our group and others has identified the 22q11 chromosomal region as potentially harboring genes for schizophrenia and Obsessive Compulsive Disorder (OCD). There is evidence in the literature to suggest that these two disorders may share some pathophysiological and genetic components. The implicated region on chromosome 22q11 spans a 1.5 megabase distance, which is amenable to positional cloning. As a complementary approach to delineate the details

of each gene's involvement, as we identify them and analyze them, we use mouse models, where the gene in question has been mutated or deleted from the animal's genome (i.e. a "knock-out" mouse). The availability of a mouse model for a gene considered as a candidate for a common, complex psychiatric disorder, although unlikely to serve as model for the entire complexity of the disorder, could provide a framework for understanding the specific nature of this gene's potential involvement.

We have generated animal models for two of the genes in the 22q11 region: (A) Catechol-O-methyltransferase (*Comt*), and (B) Proline oxidase (*Prodh*). In addition to being strong 'positional' candidate genes, *Comt* and *Prodh* are also strong 'functional' candidate genes: A) COMT along with monoamine oxidases (MAO-A and -B) are the major mammalian enzymes involved in the metabolic degradation of dopamine, norepinephrine, and epinephrine. B) The amino acid proline, although not a typical neurotransmitter, may play a modulatory role in transmission at a subset of glutamatergic synapses, a role suggested primarily by the selective expression of a brain specific high affinity proline transporter in a subset of glutamatergic pathways. Additionally, proline oxidase is involved in the biosynthesis of glutamate, and malfunction of the enzyme could affect the synthesis and release of glutamate in a specific subset of neurons.

In the case of the COMT-deficient mice, our results from microdialysis, cocaine sensitivity and behavioral assays provide conclusive evidence for an important contribution of COMT in the maintenance of steady-state levels of catecholamines in the brain and suggest a role for COMT in some aspects of emotional and social behavior in mice.

In the case of the *Prodh* gene, we isolated the mouse *Prodh* gene and identified a mutation of this gene in the Pro/Re hyperprolinemic mouse strain. Behavioral and neurochemical analysis of these mice indicated a modest deficit in sensorimotor gating (a central processing mechanism affected primarily in patients with psychiatric disorders), accompanied by neurochemical alterations in the frontal cortex and hypothalamus. The processing deficit was specific as no additional defects were observed in the amplitude and habituation of the startle response, or in locomotor and anxiety (light-dark and elevated O-maze) assays.

Since the 22q11 deletion has also been associated with specific learning disabilities, we examined the Pro/Re mice for learning deficits using the Morris water maze and the

fear conditioning paradigms. Preliminary findings based on a small number of experimental animals are largely negative.

Because the effects of genes involved in complex psychiatric disorders are thought to depend on the genetic background, we are currently extending our studies in inbred strains of mice.

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Sarah L King¹, M.B. Kelz, C. Steffen, J. Chen, E.J. Nestler and M.R. Picciotto. Trigenic mice with spatially and temporally restricted expression of high affinity nicotinic acetylcholine receptors.

High affinity nicotinic acetylcholine receptors (nAChRs) are present throughout the brain in wild type animals. Mice lacking the Beta-2 subunit of the nAChR have no detectable high affinity nicotine binding and show behavioral differences to their wild type counterparts in learning and reinforcement paradigms. Using a tetracycline regulated system we have generated mice that express the Beta-2 subunit in a regionally and temporally specific manner within the brain. The combination of different tetracycline transactivator lines crossed with one of three tetracycline-regulated Beta-2 lines allows us to generate distinct patterns of expression throughout the brain. In a Beta-2 subunit knock out background expression of high affinity nAChRs is restricted to cells expressing the transgenes. We have characterized several lines of mice showing distinct patterns of expression by equilibrium binding using the iodinated forms of the nicotinic agonists epibatidine and A85380. We have lines that express the Beta-2 subunit predominantly in the thalamus and cortex, with some expression in the hippocampus, and another line with expression restricted to a small subset of thalamic and mammillary nuclei. Characterization of other lines is in progress. Expression of these receptors can also be regulated temporally. Expression can be eliminated by treating the animals with 100 ug/ml doxycycline in the drinking water for 21 days. Testing of the transgenic animals is underway to establish the neuroanatomical substrates for nicotine's action in certain behaviors. Restoring responses to nicotine in Beta-2 knock out mice expressing the receptor in a restricted manner will allow us to pinpoint the anatomical requirements for nAChR expression in different behaviors.

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S. Leonard¹, K. Stevens, P. Bickford, L. Adler, R. Freedman. Use of rodent models of an auditory gating deficit for identification of candidate genes in schizophrenia.

The biological complexity of schizophrenia and the lack of Mendelian inheritance in this disease suggest that development of endophenotypes assayable in animals is desirable. Several animal models of traits found in schizophrenics have been developed, including prepulse inhibition, limbic cortical lesions, and gating of the P50 response to auditory stimuli. An important consideration is whether the endophenotype is a result of "state" or "trait". The inheritance of the P50 deficit has been carefully studied and found to be inherited in most schizophrenics and in approximately 1/2 of their first-degree relatives. This gating deficit, thus, represents a valid endophenotypic trait, predisposing to schizophrenia. The P50 deficit in schizophrenia also meets another criteria for thorough investigation; it can be assayed in laboratory animals where invasive pharmacological questions can be answered. We have shown that the brain circuitry regulating this trait involves a subset of the neuronal nicotinic receptor gene family, the $\alpha 7$ receptor. Nicotine, in humans, normalizes the P50 deficit. In laboratory rats, amphetamine has been used effectively to reproduce the loss of auditory gating. The induced deficit is also normalized by nicotine. The snake toxin α -bungarotoxin and methyllycconitine, both specific antagonists of the $\alpha 7$ nicotinic receptor, induce a loss in auditory gating, in the rat, similar to that in schizophrenics. A mouse model of the deficit is seen in the DBA mouse. This strain has a decrease of α -bungarotoxin-binding receptors in the hippocampus of approximately 50%, compared to the C3H strain, and exhibits a deficit in the N40 wave (similar to P50 in humans), in response to paired auditory stimuli that is normalized with nicotine. Reduction of $\alpha 7$ expression by 50%, using antisense oligonucleotides complementary to the translation start site of the $\alpha 7$ mRNA injected intraventricularly over three days, induced a loss of gating. We also found that schizophrenics had a reduction of approximately 50% in the expression of the $\alpha 7$ receptor in postmortem hippocampus compared to controls. Recent differential gene expression results, using a genechip based technology, between mouse strains with different auditory gating phenotypes indicate several other candidate genes for the behavioral deficit. These findings suggest that experiments in laboratory animals can be used

to model a sensory deficit, seen in schizophrenia, for molecular studies.

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Hans-Peter Lipp¹. Genes, brain and behavior: Bottom-up and top-down approaches².

There are two approaches to study the pathway from gene to brain to behavior. The top-to-bottom approach analyzes visible genetic variation of behavior, searches for covariates at the brain system level, and tries to identify genes causing that variation. The bottom-up (or downstream) approach follows that path from an identified (or genetically engineered) mutation, searches for covariates at the brain system level and tries to find links to behavioral phenotypes. Sometimes, structural/biochemical covariates are found yet no behavioral phenotype. The main problem of the top-to-bottom approach is to pinpoint the genes involved; the main problem of the bottom-up approach is that most targeted loci have, necessarily, pleiotropic effects on brain and behavior that are difficult to analyze fully.

I shall argue that the behavioral analysis of the consequences of targeted gene deletions can only be analyzed fruitfully if the organization of brain and behavior in the mouse is better understood. The Morris water maze will serve as an example for the (hidden) difficulties inherent in analyzing learning and memory in knockout mice. From studying more than 3000 mice in this task, we know that most mutations can express themselves only in three statistically independent factors: one factor related to wall swimming (thigmotaxis), associated with prolonged escapes times and longer swim paths; a second factor related to variations in swimming speed, and a third factor related to persistence of swimming over the old platform location in a so-called probe trial (spatial memory). Disturbingly, many mutations thought to be hippocampus-specific by superficial analysis appear to correlate with the factor thigmotaxis rather than spatial memory. But what normal processes and brain structures are responsible for prolonged thigmotactic behavior in mice? Is the hippocampus involved at all?

Classical brain lesion studies in rats are often used as reference for interpreting behavior of KO mice. As such lesion effects are often quite different in mice, we prefer a non-invasive approach, by searching for brain systems which appear to be natural regulatory sites for complex behavior. One such system identified is the mossy fiber projection in rodents, in which the (genetically variable) ex-

tent of the dentate granule cell axons synapsing on the basal dendrites of target cells (the intra/infrapyramidal mossy fiber projection, IIP-MF) appears to be linked to performance in a variety of hippocampus-dependent tasks. Using the natural genetic variation of the IIP-MF as a marker for hippocampal function, we and others have found that the hippocampus appears to be involved in mediating a much wider range of behaviors than commonly assumed, including paw lateralization and inter-male aggression. To test whether the mossy fiber system is indeed a natural regulation site for complex behavior, we have studied natural selection of mossy fiber variation in mice transferred to outdoor pens, and found significant and heritable changes after 2-4 generations. We also observed remarkable between species variation of this trait suggesting that part of the behavioral adaptation to ecological niches is achieved by natural selection of the IIP-MF.

By checking the degree of Morris water maze thigmotaxis in relation to the IIP-MF projection in a sample of mice tested a long time ago, a negative correlation between IIP-MF and thigmotaxis was found. This relation was then verified experimentally in two samples of wild voles with strongly different IIP-MF projection. Indeed, the species with the very small IIP-MF projection showed a high level of thigmotactic behavior (and no signs of spatial memory as revealed by probe trials).

Hence, by combining a bottom-up approach based on analysis of KO-mice with a top-to-bottom approach in the laboratory and the field, we can conclude that variations of thigmotaxis probably reflect an unrecognized additional property of hippocampal circuitry, namely stabilization of ongoing complex behavioral patterns (presumably reflecting stability of complex patterns of electrical activity). This property is probably not specific for hippocampal neurons. However, the hippocampus has a critical position in linking proximally connected neocortical association areas with hypothalamus, mesolimbic structures, reticular formation and associative thalamus. Thus, many non-specific impairments of the fore-brain are likely to result in a "primus inter pares" syndrome, namely (seemingly) hippocampus-dependent impairments of cognitive flexibility and memory problems in complex tasks, and many localized deficits which are not discovered by the commonly used tests to assess cognition in mice. As cognitive flexibility and stability is perhaps as (or even more) important than spatial memory to understand human intelligence and mental retardation, it

would seem mandatory to understand the underlying brain mechanisms in mice.

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**H.-P. Lipp¹, W.E. Crusio², and D.P. Wolfer^{1,3}.
Simple experimental solutions to the genetic background and flanking gene problems.**

It has been noted (Gerlai, TINS 19:177-180, 1996) that in commonly used breeding schemes mice carrying targeted mutations generally differ from littermate control animals not only by the targeted locus, but also in the origin of any genes flanking that locus. Thus, differences in flanking genes may be responsible for or contribute to the phenotype of mutant animals. We have proposed that such flanking allele effects can be tested by appropriate crosses between mouse lines backcrossed to C57BL/6 and inbred wildtype 129/J mice (Soc. Neurosci., Abstr. 24: 1203, 1998). Here, we demonstrate this experimentally using a recessive mutation (APP δ/δ , Cell 79: 755-765, 1994) which had revealed, during initial testing of conventional F₂ hybrids derived from chimeras, distinct phenotypic differences in body weight, grip strength and water maze learning. The mutation was backcrossed for 10 generations to C57BL/6. Crossing these congenics with wildtype inbred 129/J resulted in F₁ animals that were heterozygous/wildtype for the targeted locus but homozygous/heterozygous for the flanking allele segment. Phenotypically, these groups were not different, indicating lack of flanking allele effects. In order to test for confounding effects of the C57BL/6 derived chromosomal segment in the former control wildtypes and for persistence of the mutation effect, the F₁ mice heterozygous for the targeted locus were crossed again, resulting in F₂ mice that were wildtype, heterozygous or homozygous for the targeted locus but all homozygous for flanking 129/SvEv alleles. The previously observed phenotypic differences reappeared in this F₂ generation. From these observations we conclude that the initially reported phenotype was due to the targeted mutation and not to flanking allele effects. As this control experiment can be done already after 4-5 generations of backcrossing, it offers a convenient tool to verify mutation effects after the initial phenotypic assessment of conventional F₂ mice, provided backcrossing has been initiated immediately after obtaining the first germ-line chimeras.

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Benoît Martin¹, Patricia Zerr^{2,3}, and John P. Adelman². The murine *Bis1* seizure gene and the *Kcnab2* gene encoding the β -subunit of K^+ channel are different.

The convulsant methyl- β -carboline-3-carboxylate (β -CCM) is a component of the β -carboline family that has behavioral effects such as inducing seizures by acting as an inverse agonist of the GABA-benzodiazepine complex receptor. In mice, the genetic mechanism regulating seizing due to β -CCM is considered as highly multigenic with a strong epistatic component. JE/Le is a linkage testing strain carrying the *je* gene located on the distal part of chromosome 4 and maintained with forced heterozygosity. Previous work showed that JE/Le*je/je* is highly susceptible to β -CCM-induced seizures whereas JE/Le*je/+* is not. The *Kcnab2* gene encoding a cytoplasmic β -subunit (Kv β 2) modulates the pore forming α -subunits of voltage-activated potassium channels. This gene represents a strong candidate for *Bis1* since the involvement of potassium channels in epileptic processes has been clearly demonstrated and *Kcnab2* is located in the 3.1 cM confidence interval containing *Bis1*. The goal of this work is to address the question whether *Bis1* is *Kcnab2*. To this end, we have sequenced the *Kcnab2* coding sequence from mRNA extracted from JE/Le*je/je* and JE/Le*je/+* considering that they are representing respectively JE/Le *Bis1/Bis1* and JE/Le *Bis/+*. If our hypothesis is correct, we would expect different sequences for *Kcnab2* between these two populations. The only differences in the two *Kcnab2*^{je} and *Kcnab2*⁺ coding sequences, were two conservative polymorphisms. This result does not support the hypothesis that *Bis1* and *Kcnab2* are the same gene. However, it remains plausible that *Kcnab2* is implicated but this cannot be shown with JE/Le since there is no polymorphism for *Kcnab2* in this strain.

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Tsuyoshi Miyakawa¹ and Jacqueline N. Crawley. Image analysis software for behavioral phenotyping of mutant mice.

Automated quantitation of behaviors is necessary for accuracy, efficiency, and lack of bias in behavioral experiments. Here we present an advance in image analysis for mouse behaviors, which utilizes software based on a Macintosh public domain program, NIH Image. Applications were specifically designed for several commonly used tasks, including the Morris water maze, contextual and cued fear conditioning, the Barnes circular maze, an 8-arm radial maze, an elevated plus maze, open field activity, and home cage activity monitoring. Comparisons will be presented on data obtained from standard available system and the present software program, on several behavioral tasks. Features to be compared include hardware requirements, ease of setting parameters and calibration, precision and accuracy attained by 160 x 120 pixel grayscale image capture, and digital storage of images for more extensive off-line analysis. Applications of the system to behavioral phenotyping of transgenic and knockout mice will be also presented.

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D. Moechars¹, I. DeWachter, E. Godaux., B. Cordell. and F. Van Leuven. Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain.

In order to generate a transgenic mouse model for Alzheimer's disease, thirteen independent strains of transgenic mice were generated with neuronal overexpression of APP/wild type, APP/Swedish or APP/London, all resulting in essentially the same phenotype as the APP/RK transgenic mice (Moechars et al., EMBO J, 1996, 15:1265-1274), i.e. disturbed behaviour (aggression and anxiety), seizures, premature death and a disturbed glutamatergic neurotransmitter system. Differences among strains were quantitative, i.e. intensity, severity, age of onset, and directly related to transgene expression levels.

The prominent exception was the generalised occurrence of amyloid plaques only in brain of APP/London transgenic mice when more than 12 months old. Occasional plaques were observed in about half of the APP/Swedish mice, but never in APP/wt transgenic mice even when 25 months old. APP metabolites in brain were measured, i.e. total APP, secreted and membrane-bound APP, α - and β -cleaved C-terminal stubs and the 40 and 42 residue β A4 peptides. No single APP intermediate or fragment could be identified as responsible on its own, but β -cleaved C-

terminal fragments and β A4(40) peptide appeared common and essential for the phenotype. Production of β A4(42) peptide was detectable only in APP/London transgenic mice, correlating directly with amyloid plaque formation. These plaques reacted with antibodies specific for β A(40), but particularly for β A(42), were associated with hyper-phosphorylated tau and displayed other characteristics, reminiscent of plaques in AD brain.

All APP transgenic mice were cognitively impaired in Morris water-maze, already at an age of 4-6 months, which became particularly evident in transgenic F₁ offspring of FVB-C57BL crossings. Clearly, these observations dissociate in time, the early generalised cognitive deficits from the amyloid plaque formation, which is late and selective phenomenon, directly related to β A4(42) peptide formation (Moechars et al., EMBO Journal, 15: 1265-1274, 1996. Moechars et al., Neuroreport 9: 3561-3564, 1998. Moechars et al., J. Biol. Chem. 274: 6483-6492, 1999).

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Stéphane Mortaud, Laurent Nicolas, and Pierre L. Roubertoux¹. Attack behavior in mice: implication of the *sts* gene mapped on the pairing region of the X-Y chromosomes.

The sexual dimorphism of aggression has led to a search for its Y-chromosomal correlates. We have previously confirmed that initiation of attack behavior against a conspecific male is Y-dependent in two strains of laboratory mice (NZB and C57BL/6J). We have provided evidence that the nonpairing region of the Y is not involved in this behavior whereas the pairing region of the Y cosegregates with attack behavior, in these strains. In addition, the genetic correlates of attack behavior are not expressed when borne on the homologous pairing region on the X chromosome but only when carried on the Y chromosome. Only one functional gene (coding for steroid sulfatase or STS) is mapped on this region as of yet, suggesting that it could be a candidate for attack behavior. We estimated the genetic correlation between the concentration of STS protein in the liver and initiation of attack behavior. We have employed also mice in which gene invalidation induced attack behavior. Pharmacological modulations of STS or of its metabolites modifies the frequencies of attack in these male mice, confirming the implication of STS in aggression. Recent investigations have demonstrated the involvement of STS in neurosteroid

biochemical pathways, and several lines of evidence indicate that neurosteroids interact with neurotransmitters. These conclusions and our present results support the hypothesis that sulfatation of steroids may be the prime mover of a complex network, including genes shown to be implicated in aggression by mutagenesis.

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B. Olivier¹, J.A. Bouwknecht and R.Hen. 5-HT1b-knockout mice are impulsive: telemetric evidence.

Pharmacological studies using 5-HT1b receptor agonists (serenics) indicated a strong role for the 5-HT1b receptor in the modulation of aggression and impulsiveness. Because the lack of 5-HT1b receptor antagonists, no definite proof for the involvement of the 5-HT1b receptor in aggression and impulsiveness could be obtained but the availability of a 5-HT1b receptor knockout mouse opened possibilities to study these processes. We implanted male Wildtype (WT) and Knockout (KO) 129-sv-ter mice with senders and measured heart-rate (HR), core body temperature (BT) and activity (AC) on a continuous base. KO-mice reacted more to all kind of mild stressors than WT's; entering the testroom induced a higher tachycardia and a higher increase in BT and AC than in the WT. KO's were more aggressive towards male intruders than WT's. Studying their behavior in an ethological way showed that KO's are also more socially oriented and spend more time with the intruders. During aggression tests no large differences were present in the HR, BT and AC of both genotypes, but when KO's were witnessing the fights of other conspecifics they displayed, in contrast to the WT's a strong tachycardia and enhanced BT and AC. In line with previous findings we postulate that the behavioral phenotype of 5-HT1b receptor knockout mice models (aspects) of human impulsive behavior.

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J.J. Pancrazio, G.M. Grant, C.L. Wilson, D.A. Stenger, J.D. Andreadis, G.D. Ritchie, M.Y.V. Bekkedal, and J. Rossi III¹. Gene expression variability in cortical neurons:

implications for genomic in vitro prediction of neurobehavioral compromise.

The continually changing nature of operational deployment environments, and the threat of exposure to numerous chemical compounds in those environments has emphasized the importance of obtaining a capacity to rapidly predict changes in behavioral competence after exposures to unknown chemical compounds. Recent developments in array technology enable the simultaneous monitoring of thousands of genes in a single experiment. To date, gene arrays have been primarily used in pharmaceutical screening and in the comparison of normal and cancerous tissue. However, we anticipate that this approach will also prove useful in toxicological applications including the identification of novel molecular sites of action and toxicant-specific gene expression profiles, for eventual use as predictors of changes in human neurobehavioral competence. Such neurobehavioral prediction would be largely determined by the reliability of the molecular level predictors. Initial demonstrations of neurotoxicological prediction would rely on the comparison of separate populations of control and treatment cells to determine reliability in modulation of gene expression. To plan and execute statistically valid experiments, it is first necessary to evaluate the variability of gene expression between preparations of the cell/tissue system of interest. The rat AtlasTM array, commercially available from Clontech (Palo Alto, CA), was used to address the question of inherent gene expression variability among multiple cortical neuron cultures derived from E18 rats. Total RNA was isolated from cortical neurons that had been differentiated in neurobasal/B27 serum-free media for 11 days in vitro. 10 µg of RNA was converted via reverse transcriptase into [α -³³P]-dATP labeled first strand cDNA, which was then hybridized to the cDNA arrays, analyzed by a phosphorimager, and quantitated with Clontech Atlas Image software. Data from 6 culture experiments will be presented and the implications for design of studies focused on prediction of neurobehavioral compromise will be discussed.

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R. Paylor¹. Mouse genetic models for sensorimotor gating deficits.

Several patient populations with neuropsychiatric disorders, including schizophrenia, have impaired sensorimotor gating as measured using the prepulse inhibition (PPI) para-

digm. A summary of the collaborative research projects using various mouse genetic models systems to understand the genetic basis for the sensorimotor gating will be discussed. Evaluating the PPI response in different inbred strains of mice we found that sensorimotor gating is a polygenic trait. The chromosomal regions influencing PPI in the 'high' PPI responding AKR strain and 'low' PPI responding C57BL/6 strain have initially been mapped using QTL strategies. The PPI response appears to have a relatively high heritable rate of 0.65, but the preliminary QTL results detected no loci to account for a majority of the phenotypic variance. It appears that there may be several smaller (LOD score 2 to 2.5) QTLs which account for some of the phenotypic difference between the two parental strains. Gene-targeted mutant mice have also been a valuable tool for studying the role of single gene mutations in sensorimotor gating. Although many mutant mice appear to have normal PPI, we have identified the Dvl1-deficient mouse as a potential animal model system for studying schizophrenia-related traits in mice. However, in contrast to what might have been predicted, mice deficient in the α 7 nicotinic receptor subunit displayed normal sensorimotor gating. Most recently we found an abnormal PPI response in mice with a deletion of chromosome 16 in a region syntenic for DiGeorge syndrome. Mice with a deletion of chromosome 16 showed significantly greater levels of PPI compared to their wild-type controls, which is opposite of what we might have predicted since patients with DiGeorge syndrome often display 'schizophrenic-like' behaviors. We believe that using various types of mouse genetic models systems will provide us with different tools to better understand the biological basis for sensorimotor gating deficits associated with neuropsychiatric disorders.

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F. Petty¹, G.L. Kramer, and M.L. Kram. Learned helplessness and dopamine receptors.

Disturbances of mesolimbic and mesocortical dopamine function have been implied in the pathophysiology of several psychiatric disorders, including depression. Utilizing the learned helplessness animal model of clinical depression and a quantitative autoradiographic method, we studied the densities of D1 receptors and D2-like receptors in medial prefrontal cortex, septum, nucleus accumbens and caudate nucleus in rats receiving ines-

capable stress and subsequently tested for learned helpless behavior. D1 receptor densities were significantly higher in the caudate nucleus of non-helpless rats compared to learned helpless and non-stressed controls while there were no significant differences in D1 receptor density in other brain regions examined. Densities of D2 receptors were significantly lower in the core of the nucleus accumbens as well as in the medial and lateral caudate nuclei in learned helpless rats compared to the other groups. D2 receptor densities changes in the caudate nucleus may reflect a motor deficit associated with escape deficits seen in learned helpless behavior, while changes of D2 receptor densities in the core of the nucleus accumbens suggest an important role of this area in coping with the unavoidable/uncontrollable aversive stimulus. This study highlights the importance of the mesolimbic dopaminergic system in mediating behavioral responses to inescapable stress.

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I.I. Poletaeva¹, L. De Acetis², L. Ricceri³, M.G. Pleskacheva¹, N. Markina¹, A.L. Vysotski⁴, D.P. Wolfer⁴, E. Alleva², and H.-P Lipp⁴. Intermale aggression and hippocampal mossy fibers in naturally selected mice⁵.

In order to assess the effects of living conditions and the role of hippocampal mossy fiber variation on aggressive behavior of adult male mice, 19 dyads were observed in a meeting arena for five daily consecutive sessions of 10 min each. The animals had been kept socially isolated for 10 days before. All animals were derived from a mouse stock (a diallel cross involving the strains C57BL/6, C3H, NZB and DBA/2) that had underwent natural selection in outdoor pens in western Russia for two years. One member of the dyad was a mouse from the pens after two additional years of outdoor living, the other member was a descendant of outdoor pen mice that had been transferred back to the laboratory and bred there randomly for two years. The agonistic behavior was videotaped and analyzed off-line. The brains were then morphometrically analyzed for the extent of the hippocampal mossy fiber terminal fields at the mid-septotemporal level.

The feralized mice were significantly more aggressive than their opponents from the laboratory, although the level of agonistic behavior was low. The size of the intra/infrapyramidal mossy fiber projection (IIP-MF) was moderately yet significantly larger in

the mice living outdoors. However, the size of the IIP-MF projection of the outdoor living mice correlated significantly and positively with attack latencies of active mice and the frequency of freezing episodes and defensive behaviors specifically during the last encounters.

We conclude that unknown environmental and genetic factors are more important in controlling initial aggressive behavior of male mice than moderate variations of the IIP-MF; the latter, however, appear to modulate the coping style of male mice after repeated encounters rather than the level of aggression itself.

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I.Y. Ponomarev¹ and J.C. Crabbe. Genetic associations between chronic ethanol withdrawal severity and acoustic startle parameters in WSP and WSR mice².

The present study examined the genetic association between chronic ethanol withdrawal severity and acoustic startle response (ASR) in replicated lines of mice selected for high (Withdrawal Seizure-Prone; WSP) and low (Withdrawal Seizure-Resistant; WSR) susceptibility to handling-induced convulsions after withdrawal from chronic exposure to ethanol. Any differences on a nonselected (correlated) trait between the oppositely-selected lines is strong evidence of common genetic control of the selected and correlated phenotypes. In Experiment 1 naive WSP and WSR mice of both replicates were placed in startle chambers and exposed to a series of white noise stimuli of different intensities. Response habituation to a repeated acoustic stimulus was examined in Experiment 2. Results showed that WSP mice were less sensitive and more habituated to acoustic stimulation than WSR animals. In Experiment 3 WSP and WSR mice were rendered physically dependent on ethanol in inhalation chambers, and ASR parameters were measured at different time points upon withdrawal from ethanol. Both strains demonstrated a withdrawal-like pattern of decreased responsiveness to sound. No differences in the degree of the ASR reduction, however, were found between

the WSP and WSR animals. If the genetic associations reported here are verified in human subjects, reduced sensitivity and increased habituation to acoustic stimuli in naive subjects may offer predictive markers for susceptibility to ethanol physical dependence. Results of the Experiment 3 suggest that acoustic startle can be used as an independent from HIC measure of ethanol withdrawal severity in mice.

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²Supported by AA10760, AA06243, a grant from the Department of Veterans Affairs, and the Department of Behavioral Neuroscience at OHSU.

Emmanuel N. Pothos¹. Regulation of monoamine quantal size by the neuronal vesicular transporter VMAT2².

Quantal size is defined as the number of neurotransmitter molecules released by a single synaptic vesicle during exocytosis. It provides the fundamental unit of neurotransmission, which up until recently was thought to be invariant. However, this belief was based on studies of fast-acting transmitters like acetylcholine which allow small neurotransmitter overflow outside the synapse. Furthermore, this conclusion was derived from studies on postsynaptic currents, which can be confounded by the distance from the presynaptic release site and the density and affinity of postsynaptic receptors.

We adapted the use of ultrasmall (5 µm) carbon fiber electrodes to measure quantal dopamine release directly from presynaptic terminals. Neuronal stimulation evokes release of single quanta distributed in a unimodal population. Quantal release is abolished in calcium-free conditions or by reserpine and it is altered by changes in monoamine synthesis. This approach allowed the first presynaptic observation of CNS quanta, and provided the number of molecules and the duration of release during exocytosis (3,000 molecules over 200 microseconds under control conditions; Pothos et al., 1998, *J. Neurosci.* 18, 4106-4118).

Although vesicular accumulation of neurotransmitters is thought to reach a steady state based on the electrochemical gradient, it is possible that expression of vesicle transporters regulates quantal size. To examine this, we used mice with disrupted genes for VMAT2, the brain vesicular monoamine transporter, in collaboration with R. Edwards (UCSF). Although homozygous knockouts are lethal, the neurons survive in midbrain culture. Cultures derived from heterozygotes express

half normal levels of VMAT2 and also release half as much dopamine. Cultures from knockout mice have no stimulation-dependent release and markedly reduced amphetamine-mediated release. Therefore, underexpression of transporters reduces vesicular transmitter storage (Fon et al., 1997, *Neuron* 19, 1271-1283).

To examine transporter overexpression, we use both midbrain neurons and secretory cell lines (AtT-20 and PC12 cells) exposed to an adenoviral construct developed by R. Edwards. We find that overexpressors show a 4-fold increase in both quantal size and the number of vesicles released per stimulation. Furthermore, it appears that VMAT2 expression is sufficient to regulate monoamine quantal size in the absence of the tyrosine hydroxylase-dependent biosynthetic pathway, as cultured hippocampal neurons infected with the same VMAT2 viral construct become capable of stimulation-dependent monoamine release.

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L. Ricceri¹, G. Dell'Omo², D.P. Wolfer³, I.I. Poletaeva⁴, and H.P. Lipp³. Natural learning in mice: temporal and spatial adaptation of foraging behavior in a radial-maze analogue task under naturalistic conditions.

Free-living female laboratory mice, adapted to outdoor life in large pens providing a naturalistic environment, were tested for their ability to modify their foraging habits to controlled food supply. An automatic unit delivered a small portion of the daily quantity of seeds to each individual mouse. Eight of such units were placed into an outdoor pen. Mice had to visit all units to gather the daily amount of food. They were individually recognized by an implanted microchip. The number of feeding places visited every day by mice increased during a 16-days period thus showing that learning occurred in the population of the pen. At the same time, feeding activity, which previously had a trimodal profile, concentrated in an interval time around the beginning of each daily session. These data demonstrate that, despite the presence of territorial constraints and some procedural differences from lab studies, this test can be used as a radial maze analogue for field studies aimed at analyzing hippocampal functions.

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Fred O. Risinger¹. Quantitative Trait Loci for acute behavioral sensitivity to Paraoxon.

Toxicological investigations have often failed to exploit inbred strain comparisons as a research tool for the analysis of toxic mechanisms. Organophosphate (OP) compounds, particularly those used as insecticides or chemical warfare agents, have been extensively studied for at least 50 years. However, the genetic mechanisms responsible for OP-induced behavioral changes remain obscure. In the present study, provisional QTL associated with acute sensitivity or insensitivity to hypolocomotion produced by the OP paraoxon were identified. Naive adult male and female mice of the BXD/Ty series (22 different BXD strains plus C57BL/6J and DBA/2J progenitor strains) received 0 or 0.25 mg/kg paraoxon (IP), immediately before placement in an activity chamber (constructed from acrylic and aluminum, 30 x 15 x 15 cm with infrared emitter/detectors along the sides) for a 30-min test. As expected, based on previous dose-response and time course studies with C57BL/6 and DBA/2 mice, paraoxon treatment reduced locomotor activity in most, but not all BXD strains. Heritability [proportion of phenotypic variability attributed to genetic differences] was 0.58 for the paraoxon treatment effect. Difference scores were calculated for each BXD strain [strain mean for vehicle activity minus strain mean for paraoxon activity]. QTL analyses using difference scores were conducted using a database with over 1300 unique genetic markers. Several provisional QTL found on different chromosomes (i.e. 1, 2, 3, 4, 6, 8, 9, 11, 12, 13, 15) were associated with the activity phenotype. Of these, several markers attained $p < 0.01$ or greater. These were as follows: Chr 1: Ly9, $p < 0.006$; Chr 9: D9Mit15, $p < 0.003$; Chr 11: D11Ncvs76, $p < 0.002$; Chr 15: Tstap198, $p < 0.008$. Also, several markers on chromosome 3, 6 and 15 approached $p < 0.01$. Identified genes found near these regions include two plasma esterase alleles on chromosomes 6 and 9, a glutamate receptor subtype on chromosome 11 and a glycine receptor subunit on chromosome 11, raising the possibility these genes could be the basis for these provisional QTLs.

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Laure Rondi-Reig¹, Megan Libbey, Howard Eichenbaum, and Susumu Tonegawa. CA1 LTP: a synaptic mechanism of memory flexibility?

Long-term potentiation (LTP) is one relatively well-understood mechanism of synaptic plasticity which may underlie learning. In the hippocampus, the induction of LTP requires the activation of NMDA receptors. Previous studies have shown that knock-out mice with specific inactivation of the NMDA receptors in the CA1 region of the hippocampus (CA1KONMDAR1) show a CA1 LTP deficiency and are impaired in acquiring a spatial task, the Morris water maze (McHugh et al, 1996). We sought to determine whether non spatial declarative memory tasks would also be affected by this mutation. To assess this, we tested CA1KONMDAR1 mice on the transverse patterning task, a non-spatial olfactory-guided memory task. In transverse patterning, the animal must solve three concurrent discriminations (A+ versus B-, B+ versus C-, and C+ versus A-). This task is sought to require the formation of configural associations between the stimuli, and thus to be hippocampal dependent. Though this task has been shown to be sensitive to hippocampal damage in rats (Bunsey and Eichenbaum, 1996). We found that both, control and CA1KONMDAR1 mice, were able to learn the task but interestingly, CA1KONMDAR1 presented a delay in the acquisition of the task. This suggests that NMDA receptors of the CA1 region of the hippocampus are necessary to develop an efficient strategy to perform a non-spatial hippocampal dependent task.

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Richard J. Rose¹. Behavior genetics of use and abuse of alcohol.

Genetically-informative research studies of use and abuse of alcohol effectively illustrate a wide range of methodologies, problems, and promise. Although behavioral problems associated with abuse of alcohol emerge during late adolescence and adulthood, behavioral precursors, indicative of increased risk, are evident in early childhood. Children with high levels of novelty-seeking and low levels of harm-avoidance are at increased risk for development of alcohol-related problems, and as early as age three, children with a positive family history of alcoholism are behaviorally distinct from controls. But not all who are at risk choose to drink, and causal influences on initiation of drinking must be dis-

tinguished from those that affect patterns of consumption, once drinking is initiated. Initiation is primarily influenced by the drinking status of parents, siblings, and friends, and by socio-regional differences in the environments within which adolescents reside. The influence of genetic factors is negligible. But, once initiated, differences in frequency and quantity of drinking are strongly influenced by genetic factors, although genetic effects are modulated by environment and are sex-limited. Liability to alcoholism offers a compelling example of an association between a specific gene and human behavior in variation in genes coding for ADH and ALDH enzymes. But the genes that underlie alcoholism risk vary for groups with different evolutionary and cultural histories, and may be sex-limited, so molecular genetic study of alcoholism is filled with problems as well as promise. Gene-environment interaction models of the etiology of alcoholism have much potential for understanding its complex etiology.

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A.G. Sadile. The phenotypic expression of the behavioral trait of the Naples High Excitability rat-line is modified by environmental factors during early post natal life.

The aim of this study was to investigate the involvement of epigenetic factors in the phenotypic expression of the neural systems underlying attentive processes in an animal model of hyperactivity and attention deficits, the Naples High Excitability (NHE) rat-line. Male NHE pups have been reared in small (4) or normal litter size (9) during the first four weeks of postnatal life. Both groups underwent a differential handling procedure occurring 1, 2, or 4 times a week. At the end of the fourth week, rats were weaned and housed in groups of 2 and tested as young adults for non-selective attention in a spatial novelty situation for three consecutive 10-min tests at 24h intervals. The behavior was videotaped and analyzed off line for the frequency and duration of corner crossings and rearings. The results indicate that the increased maternal care and high fat diet induced by the small litter size produced long lasting effects on the duration of rearing episodes that indexes non-selective attention. Moreover, differential handling exerted a complex effect that was beneficial only at low stimulation level. In conclusion, our findings suggest an interactive role of epigenetic factors with genetic determinants during critical periods of post-natal development, thus influencing the maturation of the

neural systems controlling attentive processes.

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M. Sedelis¹, K. Hofele², I. Willuhn¹, S. Morgan¹, G.W. Auburger², J.P. Huston¹, R.K.W. Schwarting¹. Strain differences in the MPTP mouse model of Parkinson's disease: In search of the underlying genetic mechanisms³.

The application of the neurotoxin MPTP in the mouse is used as an experimental model of Parkinson's disease (PD), since it replicates behavioral deficits as well as the main biochemical and pathologic hallmarks of the disease. It is known that inbred mouse strains can differ remarkably in their susceptibility to MPTP, thus indicating a strong genetic influence. Here, we present an experimental design to approach the genetics of PD using the MPTP mouse model. In a first experiment, the inbred strains C57BL/6 and BALB/c were characterized with respect to their behavioral and neural susceptibility to systemic treatment with MPTP. One critical aim of this approach was to determine a neurotoxic dose which can be used in both sexes and strains. We found that C57BL/6 mice are more susceptible to MPTP toxicity than BALB/c mice. Effects were observed on the behavioral level as well as with respect to loss of neostriatal dopamine and cell loss in the substantia nigra. In the next and intermediate step, C57BL/6 and BALB/c mice were intercrossed to produce an F₁ generation which was analyzed in the same way as the parental strains. In these animals a reduced behavioral and neurochemical vulnerability to MPTP treatment was found. Finally, an F₂ generation was established which was treated with MPTP like the parental and F₁ animals. Our data show the relationship between the behavioral and neurochemical outcome of toxin treatment in these animals. Studying the genetic variables which are apparently critical in the mouse model may be helpful to further determine the possible genetic background in PD.

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A. Shamir¹, G. Sjholt³, R. Lovlie³, R.H. Belmaker¹, R.P. Ebstein^{1,2}, G. Agam¹, and

V. Steen³. Characterization of mouse inositol monophosphatase genes.

The enzyme inositol monophosphatase (IMPA) is a key enzyme in the phosphoinositide signaling system. IMPA is uncompetitively inhibited by therapeutically relevant concentrations of lithium (Li), a widely used mood-stabilizing medication, yet, the molecular mechanism of its effects is unknown. Berridge first proposed that the physiological effect of Li's inhibition of IMPA is depletion of brain free inositol and the consequent attenuation of neurotransmitter driven phosphoinositide second messenger signal generation. Recently we have found that the activity of the IMPA in immortalized lymphoblastoid cell lines of bipolar patients is significantly lower than those from a normal comparison group. When the bipolar patients were grouped according to clinical response to Li therapy, Li responders exhibited significantly lower IMPA activity compared to poor Li responders.

Two human IMPA genes have recently been cloned. The IMPA1 gene was found on chromosome 8q21.13-21.3 and IMPA2 was located on chromosome 18p11.2. Several studies have indicated the presence of a susceptibility locus for bipolar disorder on chromosome 18p11.2. Therefore, IMPA genes are candidates for genetic studies in bipolar disorder.

We presently report the structure of mouse IMPA-2 cDNA including the 5' and 3' untranslated regions and the genomic structure of both mouse IMPA genes. This is the first step toward the production of knockout mice lacking the IMPA genes.

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F. Sluyter¹, M. Hof¹, T. Breivik², and A.R. Cools¹. Genetic and early postnatal effects on the sensitivity to periodontitis in Wistar rats.³

Differences in the reactivity of the hypothalamic-pituitary-adrenal (HPA) axis to stressful stimuli play an important role in the outcome of the immune responses to microbial challenges. The HPA reactivity is determined by both genetic and environmental factors. Especially manipulations during the early postnatal days may lead to permanent changes. In this study we tested two hypotheses: [1] genetically selected rats characterized by high HPA-axis reactivities (abbreviated

APO-SUS) are more sensitive to ligature-induced periodontal disease than their low HPA-axis responding counterparts (abbreviated APO-UNSUS) and [2] maternal deprivation increases the susceptibility to periodontitis in APO-UNSUS rats whereas crossfostering has a decreasing effect in APO-SUS rats. The latter early postnatal manipulations have shown to affect the susceptibility to the dopamine agonist apomorphine (the original selection criterion) in these rat lines. The results show that both hypotheses are correct. In fact, maternally deprived APO-UNSUS rats show similar levels of periodontal breakdown to APO-SUS rats. Conversely, crossfostered APO-SUS rats display comparable levels as APO-UNSUS males and females. These findings indicate that both genetic and early postnatal environmental factors affect the pathogenesis of periodontitis.

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A. Smith, M. Keneshige, S.-Y. Cheng, and M.P. McDonald¹. Phenotypic analysis of mice bearing a mutant human thyroid beta receptor.

Resistance to thyroid hormone (RTH) is a heritable human condition characterized by normal or elevated levels of thyroid stimulating hormone (TSH) in the presence of high levels of serum triiodothyronine (T3) and thyroxine (T4), and resistance of pituitary or peripheral tissues to the actions of thyroid hormone. Mutations in the TR β 1 receptor gene typically result in low body weight, short stature, thyroid abnormalities, hearing loss, and mental retardation. Fifty to seventy percent of patients with RTH meet the diagnostic criteria for Attention Deficit Hyperactivity Disorder (ADHD), with the incidence about 50% higher in males than in females. ADHD is characterized by hyperactivity, inattention, learning deficits, and impulsivity. Transgenic mice bearing the human PV mutant TR β gene have been derived from a patient with severe RTH characterized by short stature, low weight, impaired learning, and ADHD, but no hearing loss. RTH transgenic mice exhibited impaired weight gain despite normal levels of thyroid hormones, TSH, GH, and IGF-1. Transgenic mice were not hyperactive but exhibited significantly more compulsive behavior on a burying task. Ongoing experiments will assess the behavior of

RTH transgenic mice on tests of learning, attention, and impulsivity.

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B.J. Snyder¹, S.P. Baron, and L.T. Meltzer. Developmental changes in the behavioral phenotype of different mouse strains².

Characterization of the behavioral phenotype of different mouse strains is becoming an important area of research. We have begun characterizing developmental differences in spontaneous locomotor activity (LMA; distance traveled and center time), acoustic startle response and prepulse inhibition of acoustic startle (PPI) in C57BL/6J (B6), DBA2/J (DBA) and 129SvImJ (129) mice by evaluation these behaviors at 6 and 12 weeks of age. At 6 weeks of age, B6 mice had the highest and 129 mice the lowest spontaneous LMA as assessed by both distance traveled and center time. The 3 strains also had different maturation patterns. The relative differences between 6 and 12 weeks were: B6 mice: no change in total distance, increase in center time; DBA mice: increase in both total distance and center time; 129 mice: no change in either total distance or center time. In terms of startle responsiveness at 6 weeks, B6, 129, and DBA mice had similar response thresholds of 100 dB. At 120 dB, B6 and 129 mice had similar magnitude startle responses, whereas DBA mice had an ~50% lower startle amplitude. At 12 weeks, the startle threshold did not change for any of the strains. However, the startle amplitude was greater for the B6 and 129 mice, whereas the DBA mice had an attenuated startle response. For PPI, Measured at 3, 6, and 12 dB over background (70dB) with a 120 dB startle stimulus, at 6 weeks, B6 mice had the lowest and 129 mice the greatest % PPI. Relative to 6 weeks, at 12 weeks, the PPI response in the 129 mice did not change, B6 mice had a greater % PPI and the DBA mice did not have any PPI response. These different behavioral and developmental patterns may influence the study of pharmacological or genetic influences on these different strain backgrounds.

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Lianne Stanford¹ and Richard E. Brown. Multivariate analyses of anxiety in inbred mice using the open field, novel object recognition and elevated plus maze².

Multivariate statistics have been used in animal behaviour for over fifty years, (J.R.

Royce, *Psychol. Bull.* 47: 235-59, 1950) and the use of these statistical methods for behavioural analysis is gaining popularity (A. Ramos and P. Mormede. *Neurosci. Biobehav. Rev.* 22: 33-57, 1998). We tested a number of mouse strains (129/Sv, p75NGFR -/-, brl/brl, brl/+, B6-H-2k & C57BL/6J) for measures of anxiety using the open field (15 measures), the elevated plus maze (17 measures) and novel object recognition (19 measures). The appropriate elements required for multivariate analyses and test battery construction are discussed. We used principal component analyses and linear multiple regression models to determine strain differences in anxiety based on the behaviours recorded in these three tests.

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Susumu Tonegawa¹. Studies on learning and memory, and activity-dependent development of the visual system with genetically engineered mice.

My lecture is composed of two parts.

A/ Multilevel analysis of hippocampus-dependent memory

One of the major goals of neuroscience is to uncover molecular, cellular, and neuronal ensemble mechanisms underlying various cognitive functions, including learning and memory. Since most cognitive functions can only be studied by analyzing performance in behavioral and cognitive tasks, it is essential to develop experimental strategies which allow the identification of the underlying mechanisms. Traditionally, pharmacological manipulations have been combined with behavioral tests. While this approach has been useful, it has certain limitations such as insufficient knowledge about the specificity of the administered compounds of the area of the brain affected by them. As an alternative method, the gene knockout technology has been introduced. For this genetic technology to be truly useful for neuroscience, it is important to target the gene manipulation to a certain area or type of cells in the brain and to be able to control its developmental timing.

Using the phage P-1-derived Cre/loxP recombination system, we have developed a method to create mice in which the deletion (knockout) of virtually any gene of interest is restricted to a subregion or a specific cell type in the CNS such as the pyramidal cells of the hippocampal CA1 region. We applied this

technique to the *NMDAR1* gene encoding the essential subunit of all known isoforms of NMDA receptors. These mice lack the *NMDAR1* gene specifically and exclusively in the CA1 pyramidal cells and only after the third postnatal week. The latter temporal restriction of the gene knockout makes it highly unlikely that any impairments observed in these knockout mice are due to a developmental abnormality. The mutant mice grow into adulthood without obvious abnormalities. Adult mice lack NMDA receptor-mediated synaptic currents and long-term potentiation specifically in the CA1 synapses and exhibit impaired spatial memory but unimpaired non-spatial memory. Our results strongly suggest that activity-dependent modifications of CA1 synapses, mediated by NMDA receptors, play an essential role in the acquisition of spatial memories. To investigate further the role of NMDA receptor-dependent CA1 synaptic plasticity in spatial memory, we studied in collaboration with Matthew Wilson place-specific firing of the mutant hippocampal CA1 pyramidal cells using multiple electrode recording techniques applied to freely behaving mice. We have discovered that although the CA1 pyramidal cells in the *NMDAR1* knockout mice retain place-related activity, there is a significant decrease in the spatial specificity of individual place fields. We have also found a striking deficit in the coordinated firing of pairs of neurons turned to similar spatial locations. Pairs have uncorrelated firing even if their fields overlap. These results demonstrate that NMDA receptor-mediated synaptic plasticity is necessary for the proper representation of space in the CA1 region of the hippocampus. The results also suggest that the reason why *NMDAR1* knockout mice are poor in acquiring spatial memory is because the lack of *NMDA* receptors in the CA1 region causes a failure in the formation of refined place fields in the CA1 and its downstream regions.

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Ruud F.G. Toonen¹, J. Peter H. Burbach, and M. Verhage. In vivo analysis of Munc18-1 protein interactions. Transgenic approach on a Munc18-1 deficient background.

Munc18-1 is a mammalian, neuron-specific member of the SEC1-family of membrane-trafficking proteins implicated in secretion of synaptic vesicles. Recently, Munc18-1 knock out mice have been generated in our laboratory. Munc18-1 deficient mice are paralysed, die postnatally and suffer from brain

degeneration. Although docked synaptic vesicles in presynaptic terminals are observed, these mice suffer from a complete and general absence of synaptic activity. Therefore, munc18-1 is an essential effector in synaptic secretion acting downstream of synaptic vesicle docking. Different aspects of this drastic phenotype may be attributed to interactions with different gene families. Munc18-1 interacts with at least 3 protein families at the synaptic nerve terminal: Syntaxin1, DOC2A/B and X11/MINT. We have generated a large number of Munc18-1 mutations characterized by selective changes in interactions with its binding partners i.e., loss of affinity for one while preserving other. These mutants enable us to establish the importance of each protein interaction. We have created transgenic mice that express Munc18-1 and certain crucial mutants in brain using three different brain specific promoters, the neuron specific enolase (NSE) promoter, the neurofilament light chain (NF-L) promoter and the human Thy-1 promoter. The NF-L promoter failed to drive expression, while the NSE and Thy-1 promoters produced neuron specific expression early in development. Overexpression of Munc18-1 does not have obvious effects on mouse behavior. Founder mice of both lines do express the transgene at birth and have been mated with Munc18-1 heterozygotes in order to obtain transgene positive Munc18-1 knockout mice. Transgenes carrying mutations in Munc18-1 known to effect the binding preference towards its binding partners are currently being analyzed.

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T. Tully¹. Genetic basis of memory.

To understand the biological basis of memory, a neurogenetic perspective asks, "What genes in the genome, when mutated, can produce learning/memory disabilities?" An adequate answer to this question will include the identification of (i) genes involved in molecular mechanisms of cellular plasticity, (ii) genes involved in the development of underlying neural architectures and (iii) genes involved in neurodevelopment and in the ongoing function of terminally differentiated neurons. Such a comprehensive genetic etiology of memory will lead initially to a valid biological categorization of cognitive dysfunction and finally to more effective behavioral and pharmacological therapies for memory loss.

Obviously, hundreds of genes likely will be involved in a complex, "emergent" function such as memory formation. Few genes, how-

ever, currently have been identified. Hence, efforts now must be focused on new gene discovery. To this end, *Drosophila* has become a valuable model system with which to find conserved genes involved in behavioral plasticity. Detailed studies of Pavlovian olfactory conditioning in the fruit fly have revealed behavioral properties that are quite similar to those characterized for other tasks in other vertebrate and invertebrate species. Such "functional homology" suggests common molecular mechanisms. The challenge now is to achieve a *vertical integration* of gene function with behavior manifestation via cellular and systems analyses.

Molecular identification of *Drosophila* genes involved with olfactory learning supports this notion. Disruptions of several enzymatic components of the cAMP second messenger system all produce olfactory learning/memory deficits. In particular, transgenic manipulations of the cAMP-responsive transcription factor, CREB, have revealed a molecular switch for the induction of protein synthesis-dependent long-term memory (LTM). Down-regulating this switch blocks LTM, while up-regulating this switch permits the formation of LTM with less training. This "photographic" memory in fruit flies is the first case of an experimentally induced supranormal memory in history.

CREB and cAMP signaling also are involved in several forms of behavioral and synaptic plasticity in rodents and mollusks. More generally, these observations demonstrate that regulation of gene expression underlies the formation of new long-term memories most likely via the growth of new synapses. This cascade of molecular and cellular events will be elucidated further with continued study of gene function in various animal model systems, thereby accomplishing a *horizontal integration* of biological basis of memory formation.

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C.E.E.M. Van der Zee¹, C. Jost, F. Oerlemans, M. Verheij, B. Wieringa, and A. Cools. Impaired learning behavior and hyperactivity observed in Brain Creatine Kinase (BCK)-deficient mice is emphasized in double mutants (BCK/UbCK-deficient) and in aged BCK-deficient mice.

Creatine Kinases play an important role in maintaining energy homeostasis, and their isoforms in the brain also form a PCr/CK circuit. It is unclear, however, to what extent the brain relies on phosphocreatine and the Brain-CK/Ubiquitous-CK catalytic activity.

At first glance, BCK-deficient mice did not show obvious abnormalities (they are fertile, carry and produce normal size litters, have a normal weight) when compared to their wildtype littermates. However, when assessed in a battery of behavioral set-ups, hyperactivity, lack of habituation, and cognitive impairments became apparent.

The BCK-deficient mice activity in the open field consisted of more walking, less sitting, (less grooming), and lacked the establishment of a "home-base", indicating reduced habituation. Cognitive function as assessed in a 3-day Morris water maze task showed that BCK-deficient mice learned significantly slower than wildtype controls. This was most apparent during the first day (and first half of the second day), but in the end they mastered the task as well as the control group. Some of the strategies used were swimming in large circles or spiralling along the wall, but, circle analysis revealed that the increased latency observed in the BCK-deficient animals was not due to thigmotaxis.

BCK-deficient mice did not differ from the wildtype mice in their sensorimotor performance in a rope grip test or in their motor coordination and balance on the rotarod.

A smaller group of double mutants (BCK/UbCK-deficient mice; n=5) and 10 aged BCK-deficient mice showed a more severe phenotype: spatial learning performance was impaired even at the third (last) day of the MWM task. Also, probe trial evaluation revealed that for aged BCK-deficient mice the % of time spend (swimming and searching for the -now- removed platform) in the NW quadrant did not go beyond chance level.

Concluding, lack of BCK appears to result in open field hyperactivity and impaired cognition in the MWM, which is emphasized in mice lacking both Creatine Kinases (BCK/UbCK) and in aged BCK-deficient mice; this suggests that BCK might play a role in the proper functioning of the hippocampus and cortical structures that do express BCKs.

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Douglas Wahlsten¹. Proof of a third source of individual differences in brain structure that is neither hereditary nor environmental.

Mice of the inbred strains BALB/c and 129 often suffer absence of the corpus callosum (CC) that normally connects the cerebral hemispheres. The characteristic involves incomplete penetrance, and variation within a strain is neither hereditary nor environmental.

Observations of axon growth in the embryo forebrain reveal a third source of individual differences that emerges from within the embryo itself. A developmental threshold magnifies microscopic embryonic variations into macroscopic differences in adult brains. The CC normally crosses over the hippocampal commissure (HC) that forms before the CC, but in BALB and 129 the HC is retarded, and the delay of HC formation imposes a very sharp threshold for CC formation that yields a bimodal distribution of adult CC size. Certain recombinant inbred strains between 129/ReJ and BALB/cWah1 never form any CC and show complete penetrance. Recombination of BALB/c and 129 genes causes a more extreme delay of HC formation that submerges every animal below threshold.

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A. Weller, A. Leguisamo, L. Towns, and D. Brunner¹. "Maternal environment" affects the offspring's behavior, in 5-HT-1A and -1B -receptor knockout mice.

Mice lacking the 5-HT_{1B} receptor (KO1B) are hyperactive and show slightly reduced anxiety in adulthood while 5-HT_{1A} knockout (KO1A) adults are more anxious. The influence of the "maternal environment" was studied by outcrossing and studying the F₁ offspring. KO1B and wild-type (WT) mice were mated in Experiment 1 and KO1A and WT mice were mated in Experiment 2. Maternal behavior was scored on postnatal day 4. The heterozygote (F₁) offspring's behavior was assessed on postnatal day 7 and in the elevated plus-maze at 2-3 months of age. No significant differences were evident in maternal behavior. F₁ pups reared by KO1B dams showed a significantly lower level of ultrasonic vocalization (USV) in the isolation test and stayed longer in the closed arm of the plus maze, compared to F₁ pups reared by WT dams. F₁ pups reared by KO1A dams produced more USV and stayed less time in the open arm, compared to F₁ pups reared by WT dams. Thus, the genotype of the mother significantly affected the behavior of the heterozygous pups, supporting a role for "maternal environment" in the phenotypic expression of anxiety-like behavior.

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Venetia Zachariou¹, A. Weathers-Lowin, B. Caldarone, T. George, J.P. Changeux, and M.R. Picciotto. Knock out of the high affinity

receptor for nicotine decreases sensitivity to cocaine.

The reinforcing properties of nicotine, opioids and psychomotor stimulants are thought to be mediated through the mesolimbic dopamine (DA) system. The present study investigates the role of high affinity nicotinic acetylcholine receptors (nAChRs) in the development of cocaine reinforcement, and examines some of the neurochemical changes in the mesolimbic DA system that might account for the interaction between nicotine and cocaine. 5 mg/kg is the lowest dose of cocaine able to condition a place preference in C57Bl/6 mice. Both chronic intermittent treatment with a high dose of nicotine for 7 days (0.7 mg/kg) and acute treatment with mecamylamine (1.0 mg/kg) were able to disrupt place preference to 5 mg/kg cocaine. Mice lacking the high affinity nicotinic receptor containing the b₂ subunit show decreased place preference to 5 mg/kg cocaine, and this decrease appears to result from a rightward shift in the cocaine dose-response curve as higher doses of cocaine can still result in cocaine place preference in these knock out animals. In contrast, b₂ subunit knock out mice respond to morphine similarly to wild type animals. Dopamine turnover was monitored in several brain areas using tissue levels of DA and its primary metabolite DOPAC as a measure of DA release. Wild type mice showed a decrease in DA turnover following treatment with 5 mg/kg cocaine, and this response was diminished in mice lacking the b₂ subunit of the nAChR. Induction of chronic fos related antigens by cocaine was also reduced in mutant mice compared to their wild type siblings, implying that changes in the dopaminergic system in these mice may also affect some of the long term effects of cocaine. These data indicate that the high affinity nAChR is likely to contribute to the development of cocaine reinforcement.

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