INTERNATIONAL BEHAVIOURAL AND NEURAL GENETICS SOCIETY

KNOCKOUTS & MUTANTS III: Genetically Dissecting Brain and Behavior
THIRD ANNUAL GENERAL MEETING
June 22 - June 23, 2000
Brighton, UK

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Program

Thursday, June 22, 2000

7.30 am-5.30 pm Registration

8.00-8.30 am Opening session

8.30-9.30 am Plenary Lecture. Jacqueline N. Crawley (Bethesda, MD, USA). What's wrong with my mouse? Behavioral phenotyping strategies and applications.

9.30-10.30 am Symposium. Genetics of eating disorders. Chair: David A Collier (London, UK)

10.30-11.00 am David A. Collier, Andreas Karwautz, Janet Treasure (London, UK). Gene-environment studies of anorexia nervosa in discordant sister pairs.

11.00-11.30 am Iain C. Campbell, Nigel Brown, Ann Ward and Janet L. Treasure (London, UK). Energy sensing in subjects recovered from anorexia nervosa.

10.30-11.00 am Coffee Break

11.00-12.30 am Contributed papers. Genetic analysis of complex phenotypes. Chair: Douglas Wahlsten (Edmonton, Ont., Canada)

11.00-11.15 am Fred van Leuven (Leuven, Belgium). Transgenic mouse models for Alzheimer's disease.

11.15-11.30 am P. Gorwood, F. Limosin, P. Batel, C. Boni, M. Hamon, and J. Adès (Colombes, France). The DAT1 gene is involved in severe alcohol withdrawal in male alcoholics.

11.30-11.45 am R. Gerlai, P. Pisacane, and S. Erickson (Indianapolis, IN, USA). Gene targeting and compensation: Behavioral effects of null mutations in the heregulin - ErbB system.

11.45-12.00 am Richard Lathe (Edinburgh, UK). The enteroceptive hippocampus.

12.00-12.15 am Catharine H. Rankin, Jacqueline K. Rose, Kenneth Eng, and Karla Kaun (Vancouver, BC, Canada). Genetic dissection of habituation of the tap withdrawal response in *C. elegans.*

12.15-12.30 am Dennis A. Stephenson, Julie Gilchrist, Dionne Peterson, Sherry Tuner, Corike Nuibe, and George A. Carlson (Great Falls, MT, USA). ENU induced behavioral mouse mutants which may involve APP or PrP.

12.30-2.00 pm Lunch break

2.00-3.00 pm Poster Session I

1/ M. Adrover, C. Blanco, E. Rial Verde, V. Cheli, G. Sanchez, L. Alché, E. Kornisiuk, E. Martín, A. Epstein, and D. Jerusalinsky (Buenor Aires, Argentina). Expression of NMDA receptor NR1 subunit sequences carried by a HSV-1 viral vector in rat hippocampus interfered with habituation.

2/ S.P. Baron (Ann Arbor, MI, USA). A titrating, continuous visual signal-detection operant schedule for mice.

3/ S. Viñas, S. Lewis, S. Barreau, C. Ducottet, A. Aubert, and C. Belzung (Tours, France). Strain differences in two animal models of depression : the forced swimming test and the chronic mild stress procedure.

4/ J. Adriaan Bouwknecht, Theo H. Hijzen, Jan van der Gugten, Rene Hen, and Berend Olivier (Utrecht, The Netherlands). Ethanol intake is not elevated in male 5-HT1B receptor knockout mice.

5/ Igor Branchi, Zoë Bichler, Marie-Claude Gonzalez, and Danièle Migliore-Samour (Orléans, France). YAC polytransgenic mouse, an animal model to assess possible alterations of cholinergic basal forebrain in Down syndrome.

6/ B.J. Caldarone, C.H. Duman, S.L. King, and M.R. Picciotto (New Haven, CT, USA). Fear conditioning, latent inhibition, and habituation in mice lacking high affinity nicotinic receptors.

7/ S. Chiavegatto, V.L. Dawson, L.A. Mamounas, S.H. Bora, V.E. Koliatsos, T.M. Dawson, and R.J. Nelson (Baltimore, MD, USA). Altered gene expression of central serotonin 5-HT_{1A} and 5-HT_{1B} receptors in male nNOS null mice.

8/ J.P. Hatcher, P.T. Davey, S. Bingham, P. Overend, A.A Parsons, and J.B. Davis (Harlow, UK). Vanilloid receptor-1 has a major role in thermal hyperalgesia.

9/ J.P. Hatcher, D.C. Rogers, C. Reavill, J.J. Hagan (Harlow, UK). Use of SHIRPA to investigate the behavioural phenotype of the Coloboma (cm/-) mouse.

10/ M. Hafezparast, S.J. Nicholson, A.S. Witherden, N. Bermingham, S. Ball, J. Peters, D.C. Rogers, J.E. Martin, E.M.C. Fisher (London, UK). Loa (legs at odd angles) a mouse model of motor neurone dysfunction: mapping and progress towards isolation of the causal gene.

11/ Josh Dubnau, Scott Gossweiler, Ulli Certa, Rod Scott, Clemens Broger, Martin Neeb, Jerry Yin, Jan Mous, and Tim Tully (Cold Spring Harbor, NY, USA). Functional genomics of long-term memory.

12/ A. Holmes, J.G. Hohmann, E. Yared, R.A. Steiner, and J.N. Crawley (Bethesda, MD, USA). Variability in an anxiety-related phenotype in galanin overexpressing transgenic mice.

13/ T. Hough, P.M. Nolan, J. Peters, E.M.C. Fisher, J. Martin, M. Browne, S. Rastan, L. Vizor, S.D.M. Brown, and A.J. Hunter (Harwell, UK). Clinical biochemistry screens can complement behavioural screens in mutagenised mice.

14/ Christopher Janus, David Westaway, Jacqueline Pearson, Peter St. George-Hyslop (Toronto, Ont. Canada). Impaired spatial leaning and memory in APP CRND8 transgenic mice.

3.00-4.00 pm Plenary lecture. Mario de Bono (Cambridge, England). The genetics of food-induced social foraging in C. elegans.

4.00-4.30 pm Coffee Break

4.30-6.00 pm Symposium. Behavioural-neurogenetic analysis of aggressive behaviour. Chair: Pierre L. Roubertoux (Orléans, France).

4.30-5.00 pm S.C. Maxson, A. Canastar, and C. Bishop (Storrs, CT, USA). Sex reversals (XX and XY females and XX and XY males), mating behaviors and aggressive behaviors in mice.

5.00-5.30 pm A.H. Veenema, G.A. Van Oortmerssen, A.J.H. De Ruiter, B. Bohus, J.M. Koolhaas, and F Sluyter (Nijmegen, The Netherlands). Neurobehavioral effects of the Y chromosome in wild house mice.

5.30-6.00 pm Stéphane Mortaud, Laurent Nicolas, Isabelle Le Roy, and Pierre L. Roubertoux (Orléans, France). Attack behavior in mice: implication of the *sts* gene mapped on the pairing region of the X-Y chromosomes.

6.00-6.45 pm IBANGS Business Meeting (Members only)

7.30-10.30 pm Banquet (Brighton Metropole Hotel)

Friday, June 23, 2000

8.00 am-5.30 pm Registration

8.00-9.00 Plenary Lecture. R. Bourtchouladze, T. Abel, A. Morozov, P. Nguyen, I. Muzzio, and E.R. Kandel (New York, NY, USA). Genes important for long-term memory and synaptic plasticity.

9.00-10.00 am Contributed paper session. Analysis of single-gene polymorphisms. Chair: Jacqueline N. Crawley (Bethesda, MD, USA)

9.00-9.15 am F. Cirulli, S. Capogrossi Colognesi, M. Bianchi, A. Panerai, and E. Alleva (Rome, Italy). Reduced sensitivity to morphine place conditioning in IL-6 KO mice. Interleukin-6 (IL-6) is a cytokine involved both in inflammatory responses and in brain function.

9.15-9.30 am J.M. Delabar, M. Rachidi, C. Lopes, C. Chabert, P. Roubertoux, C. Vayssettes, A.L. Delezoide, and E.M. Rubin (Paris, France). Abnormal cerebellar folial pattern in Yac transgenic mice containing a patterning gene from the Down syndrome chromosomal region-1.

9.30-9.45 am C. Dubertret, P. Gorwood, L. Gouya, J.C. Deybach, and J. Adès (Colombers, France). The haplotype relative risk method and the genes coding for dopamine receptors in schizophrenia.

9.45-10.00 am Alicja L. Markowska, Alena Savonenko, and Katrin Andreasson (Baltimore, MD, USA). Overexpression of cyclooxygenase-2 (cox-2) leads to cognitive impairment.

10.00-10.30 am Coffee Break

10.30-12.30 am Symposium. Human Neurodegenerative Diseases: Deciphering the Cognitive Impairment Dilemma Using Genetically Engineered Mice. Chair: Christopher Janus (Toronto, Ont., Canada)

10.30-11.00 am Fred van Leuven (Leuven, Belgium). Transgenic mice and Alzheimer pathology: no need for plaques or tangles?

11.00-11.30 am Paul Chapman (Cardiff, UK). Models of Alzheimer's disease: More than just messing around with genes?

11.30-12.00 am D. Westaway (Toronto, Ont., Canada). Transgenic mouse models of Alzheimer's Disease: long-haul or home-stretch?

12.00-12.30 am Christopher Janus (Toronto, Ont., Canada). Behavioural mouse models of Alzheimer's Disease: A hit-or-miss approach?

12.30-2.00 pm Lunch break

2.00-2.30 pm Poster Session II

15/ T. Lemberger, T. Mantamadiotis, O. Kretz, D. Gau, T. Steckler, and G. Schuetz (Heidelberg, Germany). Conditional mutagenesis of CREB in dopaminoceptive neurons.

16/ S. Lewis, M.A. Simoneau, S. Bailly, F. Guillou, B. Baron, and C. Belzung (Tours, France). Behaviour of mice expressing human transferin in brain.

17/ A.L. Vyssotski, G. Dell'Omo, D.L. Vyssotski, D.P. Wolfer, L. Minichiello, R. Klein, I.I. Poletaeva, and H.-P.Lipp (Zurich, Switzerland). Mice lacking the neurotrophin receptor TrkB in the forebrain show intact spatial memory but impaired behavioral flexibility in a semi-naturalistic set-up.

18/ K.L. McIlwain and R.E. Paylor (Houston, TX, USA). Effects of testing experience in a mouse behavioral test battery.

19/ Claudia F. Plappert, Peter K.D. Pilz, and H.-U. Schnitzler (Tübingen, Germany). Sensitization of the acoustic startle response in mice differs between two inbred strains and is influenced by glycine.

20/ C. Reavill, P. Nelson, J. Latcham, and J.J. Hagan (Harlow, UK). Prepulse inhibition and startle responses in dishevelled (Dvl1-/-) mice.

21/ Michael Regulski, Grigori Enikolopov, and Tim Tully (Cold Spring Harbor, NY, USA). Genetics of NO signaling in the adult *Drosophila melanogaster* brain.

2.30-3.30 Plenary Lecture. Peter McGuffin (London, UK). Heredity, Hazards and the Origins of Depressive Disorder.

3.30-4.00 pm Coffee Break

4.00-6.00 Symposium. The Cerebellum, Calcium and Behavior. Chair: M. Meyer (Martinsried, FRG)

4.30-5.00 pm H. Daniel (France). Calcium signalling in cerebellar Purkinje cells of mice lacking mGluR1 or $InsP_3R1$ receptors: Role in long-term depression.

4.30-5.00 pm J. Barski and M. Meyer (Martinsried, FRG). Adaptation and learning of motor coordination in mice depends on calbindin-D28k in cerebellar Purkinje cells.

5.00-5.30 pm S.N. Schiffmann, G. Cheron, A. Lohof, P. d'Alcantara, M. Meyer, M. Parmentier, and S. Schurmans (Brussels, Belgium). Calretinin, cerebellar network activity and motor coordination.

5.30-6.00 pm B. Schwaller (Fribourg, Switzerland). The lack of parvalbumin affects the body and the mind.

M. Adrover[1], C. Blanco[2], E. Rial Verde[1], V. Cheli[1], G. Sanchez[2], L. Alché[3], E. Kornisiuk[1], E. Martín[2], A. Epstein[4], and D. Jerusalinsky[1]. **Expression of NMDA receptor NR1 subunit sequences carried by a HSV-1 viral vector in rat hippocampus interfered with habituation.**

The NMDA receptor antagonist AP5 (icv) selectively impaired place learning without affecting visual discrimination learning (Morris et al., *Nature* 319:774, 1986). Infusion of NMDA receptor antagonists into the dorsal hippocampus after training caused retrograde amnesia of an inhibitory avoidance task (Jerusalinsky et al., *Behav. Neural Biol.* 58:76, 1992). To further clarify the participation of the NMDA receptor in learning/memory processing, viral vectors derived from Herpes Simplex Virus type-1 were constructed carrying either the sense NR1(+) or antisense NR1(-) sequences of the NR1 subunit gene of NMDA-R and the GFP gene. The expression was corroborated indirectly by GFP expression, and directly by binding experiments with a radiolabelled NMDA antagonist and by Western blots. The vectors were then infused into dorsal hippocampus of adult male Wistar rats. After three days, they were put in an open field and various behavioural parameters were observed to study habituation. The animals infused either with the vehicle, the GFP vector or the NR1(+) vector behaved as naive controls in the training session. All of them showed a significant decrease in both crossings -from one quadrant to another- and rearings; but the NR1(+) injected group showed an even higher statistically significant decrease in both crossings -from one quadrant to another- and rearings than control animals. The rats infused with the NR1(-) vectors performed as control animals in the training session, except for a diminished number of grooming; in the test session they showed no decrease in crossings but in rearings. Hence, this might be interpreted as either difficulties in completely recording the trace or as retrograde amnesia. These results allow to suggest that the NR1 subunit of the NMDA receptor in the hippocampus is directly involved in the mechanisms leading to habituation in such a way that even a slight change in the availability of this subunit interfered with some of the behavioural parameters recorded.

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S.P. Baron[1]. **A titrating, continuous visual signal-detection operant schedule for mice**.

Interest in genetic influences on behavior has increased and off particular interest have been the effects of gene manipulation on cognitive behaviors such as learning. However, disruptions of learning could be interpreted as disruptions of behaviors, such as attention, underlying and necessary for the expression of learning. The goal of the current experiments was to develop a method of measuring attention in mice. Experiments were performed in operant chambers containing three horizontally-placed nose-poke holes on one wall and an aperture on the opposite wall to which a dipper of 0.01 ml milk could be presented. To obtain milk reinforcement mice were required to poke into a hole within 2 sec of the offset of a light flash emitted from that hole. The initial signal duration of 100 ms was decreased by 10 ms upon a correct response and increased by 10 ms upon an incorrect response or omission. Signals were randomly presented from the three nose-poke holes. A session consisted of 135 trials. Stable behavior was determined during trials 20 –135. C57BL/6J mice (n=6) maintained an average stimulus duration of 20 ms (± 2) whereas 129S3/SvJ mice (n=4) maintained an average stimulus duration of 68 ms (± 18.6) (t-test; $p<0.001$ between strains). When the inter-trial interval was increased from 12 (± 3) to 20 sec (± 3) the mean signal duration maintained by the C57BL/6J mice increased to 44.5 ms (± 9) (t-test; $p<0.001$), whereas the signal duration maintained by the129S3/SvJ mice was not altered. The results of these preliminary studies are consistent with those reported with a five-choice serial reaction time task in mice (Humby et al., *EJN* 11, 1999) and indicate that the current task can be interpreted as a measure of attention and should be useful in the study of the effects of genetic manipulation on cognition.

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J. Barski[1] and M. Meyer[1]. **Adaptation and learning of motor coordination in mice depends on calbindin-D28k in cerebellar Purkinje cells.**

Previously we have demonstrated a specific type of ataxia in mice carrying a targeted nullmutation of the calbindin D28k gene which encodes a protein thought to act as a cytosolic calcium buffer in various populations of neurons (Airaksinen et al., 1997, PNAS 94, 1488-1493). This behavioral phenotype is recognized exclusively in environmental condi-

tions which are novel to the mice and require adaption of movement. It is, in contrast, impossible to distinguish genotypes by inspection when animals are moving in their standard cage environment. This ataxia can be described quantitatively by counting slips the mice make when walking along an elevated runway which is 100 cm long, 2 cm wide and which carries obstacles 0.5 cm in height every 10 cm. Using this assay, ataxia is clearly detectable already in young adult (6 week old) nullmutants and also, to a lesser extent, in heterozygous mice. The phenotype is not reflected in any gross developmental paramaters such as weight or size and appears to be largely independent of structural changes in the CNS. Reasoning that the cerebellum is likely to be involved and that cerebellar Purkinje cells are the only cerebellar neurons expressing calbindin and furthermore, belong to the few neuronal populations containing very high levels of this protein we focussed further analysis on the cerebellum. In cerebellar slices the nullmutation is accompanied by altered fast components of synaptically evoked calcium transients but is without effect on the elicited electrical response. To prove the critical involvement of Purkinje cells we have employed a conditional targeting strategy. To this end mice with a floxed calbindin allele and a transgenic line expressing Cre recombinase under regulatory control of the L7 gene were generated. Preliminary analysis of crosses homozygous for the floxed allele and heterozygous for the transgene reveal highly specific and efficient recombination. Conditional mutants still display the ataxic phenotype described above. Furthermore, they seem to profit less than wildtype animals from previous experience in the runway when retested a week later. Thus, Purkinje cells of the cerebellar cortex and their fast calcium - handling capabilities are critical for motor adaptation and possibly also learning, processes that presumably are essential for survival in natural habitats.

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R. Bourtchouladze[1,2], T. Abel[4], A. Morozov[1,3], P. Nguyen[5], I. Muzzio[1], and E.R. Kandel[1,2]. Genes important for long-term memory and synaptic plasticity.

Memory is not a unitary process, but consists of at least two systems — explicit memory (hippocampus-dependent), the memory of conscious recollection of facts and events, and implicit memory (hippocampus-independent), the memory of perceptual and motor strategies. The fact that these two major systems of memory utilize different strategies and they recruit different neural structures raises questions: Do they share the same or different cellular and molecular mechanisms? Can molecular genetics, with its ability to discern homology relationship, discern commonalities or differences in these two very different memory systems? One evidence to shared mechanisms has come from studies of stages in memory storage. Both explicit and implicit memory display at least two temporally distinct memory processes: short-term and long-term memory. Moreover, in each case, long-term memory requires new protein synthesis.

Studies in *Aplysia* and *Drosophila* provided the initial evidence that cAMP signaling pathways play an important role in implicit forms of learning in invertebrates. Here we have taken a genetic approach to explicit memory storage. We have generated transgenic with reduced PKA activity in hippocampal neurons [R(AB)-mice], and mice expressing a dominant negative small GTPase Rap1, , in the forebrain under the control of the tetO/tTA system. Rap1 mice show a reduction of coupling of the cAMP cascade to the MAP kinase signaling. We explored both transgenic mice in biochemical, physiological, and behavioral studies.

We found that PKA and Rap1 play a critical role in the hippocampus in initiating the molecular events leading to the consolidation of short-term changes in neuronal activity into long-term memory. Taken together with studies demonstrating the crucial role of CREB-induced transcription in implicit and explicit memory storage (*Aplysia, Drosophila*, and mice), our results indicate that quite different memory processes use a restricted number of mechanisms for converting short- to long-term memory.

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J. Adriaan Bouwknecht[1], Theo H. Hijzen[1], Jan van der Gugten[1], Rene Hen[2], and Berend Olivier[1,3,4]. Ethanol intake is not elevated in male 5-HT1B receptor knockout mice.

Rationale: Recently, the finding that 5-HT1B receptor knockout (5-HT1B KO) mice have increased ethanol intake could not be replicated. We assessed ethanol consumption in male wildtype (WT) and 5-HT1B KO mice derived from the original population (Crabbe et al. 1996). Objectives: To investigate whether elevated ethanol consumption is present using

the original paradigm in our colony with genetic makeup as similar as possible to the original study. Methods: Mice had continuous access to two pipettes, one filled with water and one with increasing concentrations of ethanol (0, 3, 6, 10 or 20% v/v). Fluid intake was determined daily. Results: Ethanol intake (g/kg body weight) did not differ between genotypes. However, body weights (20-25%) and water intake (50%) were consistently elevated in 5-HT1B KO mice. Conclusions: Phenotypic effects on ethanol intake could not be replicated. These data confirm other studies, suggesting ethanol intake in 5-HT1B KO mice is not increased. Hence, the initial finding of elevated ethanol intake in 5-HT1B KO mice may have been due to phenotypic differences in fluid intake.

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Igor Branchi[1], Zoë Bichler[1], Marie-Claude Gonzalez[1], and Danièle Migliore-Samour[1]. YAC polytransgenic mouse, an animal model to assess possible alterations of cholinergic basal forebrain in Down syndrome.

Down syndrome (DS) the most frequent genetic cause of mental retardation (1:700 live births), is due to an extra copy of chromosome 21. Structural and functional abnormalities in the central nervous system of DS individuals have been widely reported. The first aim of DS research in recent years has been to identify the genetic bases of the different phenotypic aspects. For this purpose, YAC transgenic mouse models of DS have been developed by inserting in the murine genome a Yeast Artificial Chromosome (YAC) bearing a fragment of the human Down syndrome Chromosomal Region (DCR). This region was reported to play an important role in the development of typical DS features, including mental retardation. In YAC mice, trisomy is due to two copies of mouse chromosome 16 and one copy of homologous fragment of human chromosome 21 included in the YAC. Several works reported a neurodegeneration of cholinergic neurons in DS brain, and, more recently, a treatment based on acetylcholinesterase inhibitors has been shown to improve cognitive abilities in DS patients. An assessment at developmental and adult phase of neurobiological and behavioral deficits in YAC mice has been carried out. Abnormalities of basal forebrain cholinergic neurons have been investigated using specific neuronal markers, and behavioral impairments have been monitored in a passive avoidance and a two-object recognition task. These analyses, performed on YAC mice bearing different fragments of the DCR, provided information to identify which part, or even gene, of such region is responsible for the different features of cognitive impairment displayed by DS subjects.

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B.J. Caldarone[1], C.H. Duman[1], S.L. King[1], and M.R. Picciotto[1]. Fear conditioning, latent inhibition, and habituation in mice lacking high affinity nicotinic receptors[2].

Although nicotine has been reported to improve performance in several tests of cognition, the specific nicotinic receptor subtypes that mediate these effects are largely unknown. As a first step in understanding which receptor subtypes regulate the cognitive effects of nicotine, baseline performance of knockout mice lacking the beta-2 subunit of the nicotinic receptor and wildtype controls was evaluated in fear conditioning, latent inhibition, and habituation tasks. In the fear conditioning task, mice were administered 3 pairings of a tone with foot shock and tested for freezing to the context and tone 24 hr later. Young (2-4 months) knockout and wildtype mice did not differ in fear conditioning, although aged (9-20 months) knockout males exhibited less freezing to the context and tone compared to aged wildtype males. No differences in fear conditioning were observed between aged knockout and wildtype females. Latent inhibition of fear to a pre-exposed tone was also assessed. Both knockout and wildtype mice displayed similar levels of latent inhibition, although overall levels of freezing were lower in knockout mice. Locomotor habituation to a novel environment did not differ between wildtype and knockout mice in either young or aged animals. These results support the previous study showing learning deficits in aged beta-2 knockout mice (Zoli et al., 1999, EMBO J., 18, 1235) and suggest that other cognitive tasks may not be influenced by beta-2 receptors.

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Iain C. Campbell[1], Nigel Brown[1], Ann Ward[1], and Janet L. Treasure[1]. Energy Sensing in subjects recovered from Anorexia Nervosa.

Leptin is a lipostatic hormone also involved in the control of metabolism, and its deficiency leads to massive obesity. We hypothesised that overactivity in this hormonal system contributes to the development of AN. In rodents, glucose metabolism is an energy sensing system and the activity of this pathway is linked to leptin production. Furthermore, in normal weight and obese subjects, leptin secretion increases in the late evening in an manner related to daily food intake indicating it is related to energy balance. Accordingly, we tested the general hypothesis that abnormalities in the glucose-insulin-leptin axis are predisposing factors for AN. 18 females recovered from an episode of AN (to avoid starvation related changes) were given a standard meal, and glucose, insulin, b-hydroxybutyrate and leptin responses, compared with age and BMI matched controls (see Ward et al, 1998). Bloods were collected at 15 minute intervals from 11:00 and then at 12:45, 13:45 and 14:45. The meal was presented at 11:15 and had to be eaten by 12:15. Subjects were allowed diet soft drink or black coffee with the meal and free access to mineral water. Recovered AN subjects showed a normal BMI-leptin correlation and, as for controls and obese subjects, showed no immediate leptin response. We observed both increases and decreases in post meal leptin responses, which may be related to the energy balance of the individual. There is an inverse correlation between changes in plasma leptin and glucose in the post meal period in the controls: we propose it is a manifestation of an energy sensing system in humans. Thus, as glucose shows small rebound increases following the post prandial hypoglycemia, leptin decreases, signalling that energy requirements are being fulfilled. Perhaps more importantly, this relationship between leptin and glucose is absent in the recovered AN group. From this, an hypothesis on the aetiology of AN can be proposed: 1) AN subjects are unable to accurately monitor the availability of body energy; 2) because they do not accurately sense levels of body energy, subjects prone to AN are able to escape from the normal homeostatic process which increases food intake when the demand for energy is increased: as a consequence they are able to deplete their energy stores and lose weight.

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P. Chapman[1]. Models of Alzheimer's disease: More than just messing around with genes?[2]

The identification and manipulation of Alzheimer's disease (AD) related genes has provided a major impetus for the creation of animal models. Not surprisingly, mouse models have led the way. Mice overexpressing several different mutant forms of amyloid precursor protein (APP), presenilin 1 (PS1) and risk factors for AD (such as ApOE) have been created, crossed and tested for histopathology ranging from amyloid plaque deposition to cell and synapse loss. Because AD is characterised in humans by loss of cognitive function, the generation of tests for the behavioural and physiological consequences of AD-related gene mutation is both critically important and challenging. We have analysed both mice and rats overexpressing mutated (670-671NL) human APP, examining learning, memory and attention while also measuring synaptic physiology. Aged transgenic mice and rats both demonstrate behavioural impairments, though the severity is greater in the mice, either as a result if increased gene dosage, greater susceptibility, or both. On the other hand, the range of behavioural tests that can be applied to rats suggest that future attempts to characterise their deficits may produce a greater understanding of the nature of behavioural processes affected by AD-related gene mutations than would be possible by studying mice alone. Aged transgenic mice also demonstrate altered synaptic physiology, being more sensitive to trauma, more excitable, and less likely to demonstrate long-term synaptic enhancement. The opportunity to conduct long-term recordings from chronically implanted rats will be of great value in correlating the physiological and behavioural consequences of AD.

[1]Cardiff School of Biosciences, Cardiff University, Cardiff, UK. [2]This work was supported by grants from the Wellcome Trust and the U.S. National Institute of Aging (NIA).

S. Chiavegatto[1], V.L. Dawson[1], L.A. Mamounas[1], S.H. Bora[1], V.E. Koliatsos[1], T.M. Dawson[1], and R.J. Nelson[1]. Altered gene expression of central serotonin 5-HT_{1A} and 5-HT_{1B} receptors in male nNOS null mice.[2]

Male mice with targeted disruption of the neuronal isoform of nitric oxide synthase (nNOS) display a marked increase in aggressive behavior with no difference in blood testosterone concentrations. Several lines of research have established a role for brain serotonin (5-HT) in aggression. Studies using selective 5-HT receptor agonists and genetically engineered mice strongly suggest that 5-HT_{1A}

and 5-HT_{1B} receptors play important roles in aggression. We investigated the neurochemical profile of these mice. Serotonin turnover (5HIAA/5-HT) was reduced in the cortex (18.1%), hypothalamus (18%) and midbrain (16.4%) of $nNOS^{-/-}$ mice ($p<0.05$). We thus examined the 5-HT terminals by immunocytochemistry. There was no significant alteration in the density and pattern of 5-HT terminals in the $nNOS^{-/-}$ mice suggesting that the selective disturbance in the serotonergic system in the brain of $nNOS^{-/-}$ mice is not due to a loss of 5-HT axons or structural alterations. We used a semi-quantitative RT-PCR methodology and focused on the mRNA levels of four 5-HT receptors 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} implicated in aggression/impulsivity. The mRNA levels of the 5-HT receptors were normalized according to the mRNA levels of GAPDH in each brain area. The expression of postsynaptic 5-HT_{1A} receptors was dramatically decreased in hypothalamus (49 ± 4%) and increased in both the hippocampus (41 ± 6%) and amygdala (94 ± 34%), whereas the mRNA level of 5-HT_{1B} receptor was decreased in frontal cortex (35 ± 8%) and increased in the hippocampus (39 ± 5%) in $nNOS^{-/-}$ mice. The expression of the postsynaptic 5-HT_{2A} and 5-HT_{2C} receptors was similar between WT and $nNOS^{-/-}$ mice in all brain areas studied. In the midbrain, the mRNA of 5-HT receptors and the mRNA of tryptophan hydroxylase (5-HT synthetic enzyme) and 5-HT transporter were not different between genotypes. These data suggest that selective disturbance of the 5-HT_{1A} and 5-HT_{1B} receptors expression might be responsible for the aggressive phenotype of $nNOS^{-/-}$ mice.

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F. Cirulli[1], S. Capogrossi Colognesi[1], M. Bianchi[2], A. Panerai[2], and E. Alleva[1]. Reduced sensitivity to morphine place conditioning in IL-6 KO mice. Interleukin-6 (IL-6) is a cytokine involved both in inflammatory responses and in brain function.

In this experiment the positively-reinforcing properties of IP morphine were assessed using a place conditioning paradigm in transgenic male mice not expressing IL-6 (IL-6 KO) and in control (IL-6 +/+) subjects. Following an habituation session, IL-6 KO mice and their wild-type littermates were exposed to a conditioning procedure, each mouse receiving four pairings of morphine (10 mg/kg) with specific environmental cues (floor texture, wall colour). The testing apparatus consisted in one white and one black compartments communicating through a neutral (grey) zone. Morphine injection was always associated to the white compartment. Conditioning trials lasted 30 min each and the experiment ended with a 15 min preference test performed in a drug-free state. Significant differences in time spent in the different compartments of the apparatus between KO and wild-type littermates emerged during the habituation session. Specifically, IL-6 KO mice spent more time in the white compartment, while controls spent most of the habituation session in the black area of the apparatus. On the test session, significant differences between IL-6 null mice and their controls were no longer evident since this last group spent significantly more time in the white compartment, compared to pre-conditioning levels. No conditioned place preference was shown by IL-6 KO mice, in line with previous reports showing a reduced sensitivity to the analgesic effects of morphine in these null mice and reduced density of mu receptors. Overall these data indicate that IL-6 appears involved in the expression of emotional behaviours and in responding to exogenous opiates.

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David A. Collier[1], Andreas Karwautz[1], Janet Treasure[1]. Gene-environment studies of anorexia nervosa in discordant sister pairs.

Previously, a number of investigators have implicated the 5-HT2A gene as an aetiological factor in anorexia nervosa. In order to clarify the role of this gene, we examined both genetic and environmental risk factors for AN using a within family, case-control design of 45 discordant sister pairs. The anorexia nervosa phenotype was associated with the personality traits of harm-avoidance, persistence, perfectionism, ineffectiveness as well as several axis I & axis II disorders. Novelty seeking, interoceptive awareness and self-directedness were all reduced. The sisters with AN differed from their healthy sisters in terms of personal vulnerability traits and exposure to high parental expectations and sexual abuse. Factors within the dieting risk domain were not significant, but instead there was evidence of poor feeding in childhood. We found no difference in the distribution of genotypes or alleles of the DRD4, COMT, the 5HT2A, and 5HT2C receptor genes. However, these results have to be seen as preliminary because our power calculations indicate that there is insufficient power

to detect the expected effect on risk with the sample size employed.

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Jacqueline N. Crawley[1]. What's wrong with my mouse? Behavioral phenotyping strategies and applications.

Targeted gene mutation presents a powerful new tool for understanding the role of genes in behavior. Rigorous experimental design is required for the behavioral phenotyping of transgenic and knockout mice. Use of well established, quantitative behavioral tasks, appropriate Ns, correct statistical methods, consideration of background genes contributed by the breeder parents, and consideration of litter and gender issues, will yield meaningful comparisons of -/-, +/-, and +/+ genotypes.

Our laboratory attempts to design methods to optimize the behavioral characterization of mutant mice. Initial observations evaluate general health, neurological reflexes, sensory abilities, and motor functions. Specific tests include measures of home cage behaviors, body weight, body temperature, appearance of the fur and whiskers, righting reflex, acoustic startle, eye blink, pupil constriction, vibrissae reflex, pinna reflex, Digiscan open field locomotion, rotarod motor coordination, hanging wire, footprint pathway, hot plate tactile response, visual cliff, and acoustic startle.

Hypothesis testing then focuses on at least three well-validated tasks within each relevant behavioral domain. Specific tests will be described and illustrated for the domains of learning and memory, feeding, nociception, and behaviors relevant to discrete symptoms of human anxiety, depression, schizophrenia, and drug addiction. Adaptation of standard rat tests for use in mice, and development of new tasks for mice, are necessary in many behavioral domains.

An example of our approach will be described for the behavioral phenotyping of galanin overexpressing transgenic mice, generated in the laboratory of our collaborator Robert Steiner at the University of Washington in Seattle, as a model for the striking galanin overexpression in Alzheimer's disease. Galanin transgenic mice were normal on measures of general health, reflexes, sensory and motor abilities. As compared to wildtype littermates, galanin transgenics displayed impairments on cognitive tasks, supporting the growing literature that galanin is an inhibitory modulator in cholinergic pathways relevant to learning and memory.

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H. Daniel[1]. Calcium signalling in cerebellar Purkinje cells of mice lacking mGluR1 or $InsP_3R1$ receptors: Role in long-term depression.

Activation of subtype 1 metabotropic glutamate receptors (mGluR1) and subtype 1 inositol 1,4,5-trisphosphate receptors ($InsP_3R1$) play a key role in the induction of long-term depression (LTD) of synaptic transmission at parallel fiber (PF)-Purkinje cell synapses in the cerebellar cortex, as confirmed by impairment of this form of plasticity in mGluR1 deficient transgenic mice (Conquet et al., 1994) and in $InsP_3R1$ deficient transgenic mice (Inoue et al., 1998). Activation of mGluR1 stimulates intracellular cascades, involving protein kinase C (PKC) activation and calcium release from $InsP_3$-sensitive internal stores through the production of $InsP_3$. Indeed, both of these cascades, at least in certain experimental conditions, are likely to be involved in LTD induction.

The aim of the present study was to investigate in *in vitro* thin cerebellar slice preparations of these mutants and wild-type mice, possible interactions between various sources of calcium in dendrites of Purkinje cells, that can be involved in the induction of LTD. In addition to the patch-clamp recordings of PF-mediated EPSPs in current clamp mode, cytosolic free calcium concentration $[Ca^{2+}]_i$ in proximal of wild-type and mutant Purkinje cells was measured with fluorescence images recorded with a-CCD camera, or with fluorometric method with a photomultiplier detector system. This was achieved by dialysing the calcium sensitive dyes fluo-3 (100 μM) or bis fura-2 (100 μM) into Purkinje cells through the patch-clamp electrode.

In mGluR1 deficient Purkinje cells, the calcium signal from mGluR-InsP3 pathway was absent, whereas the calcium responses due to direct activation of voltage-dependent calcium channels and to release from ryanodine-sensitive stores were at least qualitatively preserved. In addition, the lack of functional mGluR1 did not alter, at least qualitatively, the $InsP_3$-dependent control of calcium release from internal stores, since it was possible to detect $[Ca^{2+}]_i$ dendritic changes induced by photolytic release of 25 μM $InsP_3$. In these mutants, the combination of calcium influx through voltage-dependent calcium channels and calcium release from $InsP_3$-sensitive internal stores following photolytic release of $InsP_3$ (but not from ryanodine-sensitive internal stores) rescued a PKC-dependent LTD.

This demonstrates that impairment of LTD in mGluR1-deficient mice is not due to major abnormalities in the signal transduction pathways involved in LTD induction dowstream mGluR1, but is solely due to the lack of functional mGluR1.

In Purkinje cells from mice with a disrupted $InsP_3R1$ gene, calcium responses due to direct activation of voltage-dependent calcium channels were preserved. Application of a selective agonist of mGluR (1S,3R-ACPD) induced no variation of $[Ca^{2+}]_i$ in dendrites and soma, indicating that in these cells, calcium release from internal stores does not occur after $InsP_3R1$ activation. Nevertheless, in some mutant cells, clear increases in dendritic and somatic $[Ca^{2+}]_i$ were detectable during 1S,3R-ACPD application, but these rises appeared to reflect calcium influx via voltage-gated calcium channels, because they were temporally associated with spike firing. Finally in these cells, the release of calcium from ryanodine-sensitive stores was preserved since caffeine, a ryanodine receptor agonist, first caused reliable and reversible elevation of $[Ca^{2+}]_i$ in dendrites and soma, and second increased calcium signals evoked by cell depolarization.

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M. de Bono[1]. Genetics of natural variation in C. elegans social behaviour.

Successful foraging is a pre-requisate for the evolutionary success of a species. Different natural isolates of *C. elegans* exhibit distinct foraging patterns in response to food. Animals from social strains aggregate and feed together on a lawn of bacteria. Animals from solitary strains show no aggregation and feed in isolation. About one third of wild isolates are solitary and two thirds are social. Variation at a single major genetic locus, *npr-1*, is responsible for this natural difference in foraging. *npr-1* encodes a potential seven transmembrane domain neuropeptide receptor with two natural variants that correlate with social and solitary behavior. The two variants differ at a single amino acid position that may alter receptor activity.

Social foraging occurs in response to signals derived from food. To understand how this behavior is generated we are defining the food signals that elicit social behavior, and the molecular and cellular signaling pathways that positively and negatively regulate social foraging. Studies of previously identified *C. elegans* mutants suggest that social foraging is regulated by pathways that are genetically distinct from those controlling other *C. elegans* behaviors. To identify components of these pathways we have conducted screens for mutations which abolish social behavior. Most of the mutants identified thus far appear behaviorally normal, but forage in isolation.

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J.M. Delabar[1], M. Rachidi[1], C. Lopes[1], C. Chabert[2], P. Roubertoux[2], C. Vayssettes[1], A.L. Delezoide[3], and E.M. Rubin[4]. Abnormal cerebellar folial pattern in Yac transgenic mice containing a patterning gene from the Down syndrome chromosomal region-1.

Down syndrome is the major cause of mental retardation in humans. A considerable portion of the Down syndrome pathology has been mapped to the Down syndrome chromosomal region-1 (DCR-1) in 21q22.2. A library of Tg mice has been recently constructed with Yac clones from the DCR-1 (Smith et al 1997). In this study the neuropathological analysis of two lines carrying one copy of the Yac 230-E8 (650kb) reveals a major modification of the cerebellar folial pattern: lobules III to VI are clearly larger than normal; this hypertrophy increases the size of these lobules by 1.3-1.7x. Granule cell density and layering appear to be normal in the cerebellum. Among the 5 known genes that contains the Yac 230-E8, one, C21orf5, has two orthologs involved in patterning (in C.elegans and in drosophila). This gene is strongly expressed in frontal cortex, hippocampus and cerebellum of human (normal and DS) and mouse embryos. A similar expression pattern is observed in normal and Tg mice with human and mouse probes respectively. These observations suggest that C21orf5 could play an important role in the patterning of the cerebellum and in the pathogenesis of Down syndrome.

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C. Dubertret[1], P. Gorwood[1], L. Gouya[1], J.C. Deybach[1], and J. Adès[1]. The haplotype relative risk method and the genes coding for dopamine receptors in schizophrenia.

The involvment of dopamine in the aetiology of schizophrenia is suggested by number of neurobiological and pharmacological data. The role of the different dopamine receptors in schizophrenia is remaining unknown, as association studies showed conflicting re-

sults. We thus reexamined the dopamine hypothesis controling for one of the major bias of association studies (i.e. statification bias) and one of the major limitation of parametric linkage studies (i.e. estimating the unknown characteristics of the type of inheritability), using the haplotype relative risk method. We searched for non-randomly transmitted alleles at the *DdeI* DRD1, *Taq1D* DRD2, *Bal1* DRD3, *FspI* DRD4, DRD5 microsatellite and (TCAT)n repeat TH loci. These polymorphisms were tested in 37 trios, containing the schizophrenic proband and both parents. Our results do not support a major role of *Taq1D* DRD2 (c^2=1.09, df=1, p=0.30), *Bal1* DRD3 (c^2=0.03, df=1, p=0.86), DRD4 (c^2=0.24, p=0.62), DRD5 microsatellite (c^2=, df=11, p=0.89), and (TCAT)n repeat TH (c^2=0.47, df=4, p=0.98) polymorphisms. We found a significant excess of transmission of the allele 1 (c^2=3.69, df=1, p=0.05), with a closed-to-significant excess of genotype 1*1 at the DdeI DRD1locus (c^2=5.29, df=1, p=0.07). The present analysis suggest a small but significant effect of DRD1 gene in the susceptibility to schizophrenia, but the sample size needs to be increased before concluding for evaluation its real impact.

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Josh Dubnau[1], Scott Gossweiler[1], Ulli Certa[1], Rod Scott[1], Clemens Broger[1], Martin Neeb[1], Jerry Yin[1], Jan Mous[1], and Tim Tully[1]. Functional genomics of long-term memory.

Three features of long-term memory are conserved across animal phyla. First, memories are initially stored in a short-term labile form but can progress to a longer lasting, stable form. Second, long-term but not short-term memory requires a new program of gene expression. And third, many tasks require repeated training sessions interspersed with rest intervals (spaced training), rather than repeated training without rest intervals (massed training), to produce long-term memory. Numerous attempts at identification of genes and pathways that are induced during memory consolidation have so far focused either on in-vitro models of neuronal plasticity or in-vivo pharmacological manipulations of neuronal activity. These studies have led to a laundry list of genes that likely play important roles in neuronal plasticity in a broad sense. In contrast, only a handful of transcripts and proteins have been discovered that are induced or repressed during memory consolidation per se. Hence identification of genes involved specifically with long-term memory has been difficult.

In Drosophila, spaced training results in several short-term forms of memory, as well as in long-term memory, which is CREB- and protein synthesis-dependent. In contrast, memory after massed training is less stable, CREB independent, and insensitive to protein synthesis inhibitors. We have used these behaviorally specific training protocols and mutations that disrupt memory, in combination with Affymetrix gene chip technology, to characterize a genomic response to memory formation. We have identified several memory candidate genes.

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R. Gerlai[1,2], P. Pisacane[1], and S. Erickson[1]. Gene targeting and compensation: Behavioral effects of null mutations in the heregulin - ErbB system.

Genetic redundancy is a problem in gene targeting studies because functionally relevant sister proteins can compensate for the lack of protein product of a targeted gene. We chose a molecular system in which we hope to demonstrate both the lack and presence of compensation after dysruption of particular single genes. Mammals may not be able to compensate for the lack of heregulin, a single ligand for multiple ErbB receptors, however, compensation is expected when a single ErbB receptor is knocked out. To investigate this, we disrupted the heregulin-1 , ErbB2, or ErbB3 locus in a targeted manner and analyzed mice heterozygous for the mutation. Heregulin and its receptors were shown to be involved in embryonic brain development and, more recently, in plastic changes associated with adult brain function in rodents. Although they have never been shown to play roles in mammalian behavior, we decided to characterize the mutant mice behaviorally using a battery of simple tests. Despite the absence of gross morphological defects, heregulin mutant mice exhibited elevated activity levels in the open field, showed improved rotorod performance, and finished T-maze spontaneous alternation task faster compared to control wild type littermates, findings that suggest a consistent hyperactivity across tests. ErbB2 and ErbB3 mutant mice, whose strain origin was identical to that of heregulin mutants, showed no sign of the behavioral alterations. We suggest that the abnormalities seen in heregulin mutant mice were due to mutation at that locus and the lack of alterations seen in ErbB2 and ErbB3 mutant mice is the result of compensation by unaltered sister receptors.

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P. Gorwood[1], F. Limosin[1], P. Batel[1], C. Boni[1], M. Hamon[1], and J. Adès[1]. The DAT1 gene is involved in severe alcohol withdrawal in male alcoholics.

Introduction. Two German case-control studies (Sander et al., 1997 ; Schmidt et al., 1998) showed that the A9 allele may increase the risk for severe withdrawal symptoms for alcohol-dependent patients who stop drinking (specially withdrawal seizures and delirium). These studies were consistent as one was retrospective and qualitative, and the other prospective and quantitative. We analyzed lifetime withdrawal symptoms in 120 French alcohol-dependent patients, in order to specify which symptoms and/or patients may be more specifically involved in complicated alcohol-withdrawal symptoms. *Method*. Patients were assessed with the DIGS (Diagnostic Interview for Genetic Studies) for lifetime psychiatric and addictive disorders. Expected allele frequencies were based on 65 control subjects without psychiatric or addictive morbidity, and matched for male gender and French origins. The A9 allele of the DAT1 was revealed with the previously described PCR. *Results*. In our sample, the A9 allele was more frequent in patients who had at least once withdrawal seizure or delirium (p=3D0.028), showing the same trend as did the first study. There was a significant linear trend for increased number of withdrawal symptoms in patients with the A9 allele (p=3D0.03). Furthermore, patients who took alcohol at least once in order to reduce withdrawal symptoms were more frequent in the group of patients who have the A9 allele (p=3D0.027). Although the distribution of symptoms is not superimposable for the two groups (with versus without the A9 allele), no specific symptom (or clinical group of symptoms) was found increased in one group. We also performed a factorial analysis in order to detect a role of the DAT gene on latent classes. The factorial analysis of the 120 patients showed the existence of 3 factors, explaining 55% of the total variance. It is noteworthy that the DAT1 gene was associated only in one specific dimension, untitled "severe neuropsychiatric withdrawal symptoms". Interestingly, the frequency of the A9 allele (54.0%) in the group of 65 healthy controls (without alcohol abuse or dependence) matched for sex and origins was intermediate between alcohol-dependent with withdrawal complications (71.4%) and those without such symptoms (42.3%), addressing the question whether the A9 allele is a risk factor or the A10 allele is a protective factor against complicated withdrawal. *Conclusions*. The role of the A9 allele of the DAT gene in withdrawal complications is replicated in this independent sample, probably involving severity of symptoms rather than a specific type of symptom (seizures and delirium are generally considered as the most severe symptoms of withdrawal).

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M. Hafezparast[1], S.J. Nicholson[1], A.S. Witherden[1], N. Bermingham[1], S. Ball[2], J. Peters[2], D.C. Rogers[3], J.E. Martin[4], E.M.C. Fisher[1]. Loa (legs at odd angles) a mouse model of motor neurone dysfunction: mapping and progress towards isolation of the causal gene.

Motor neurone diseases (MNDs) are neurodegenerative disorders that kill 1 in 500 adults in UK. Some of these diseases have genetic aetiology. For example, approximately 20% of cases of amyotrophic lateral sclerosis (ALS) are familial and so far only one gene, superoxide dismutase 1 (SOD1), has been identified in 10-15% of familial ALS. Thus the majority of the genes involved in the pathology of MND remain to be identified.

We have a mutant mouse, called legs at odd angles (Loa), that exhibits an autosomal dominant motor function loss in the hind limbs. Homozygotes for the Loa die within 24 hours of birth. However, the gross anatomy of the progeny is apparently normal, indicating normal development. Heterozygous Loa/+ mice on the other hand are viable, have a normal life span, and can be identified by a characteristic clasping of the hind limbs when suspended by the tail. Compared with their wild type littermates, these mice perform significantly more poorly in the rotarod test of balance and coordination, but show increased spontaneous locomotor activity. These features of Loa mice are more profound in older animals, indicating that the Loa mutation results in a progressive motor function deficit. Histopathological analysis has revealed a significant decrease in the number of anterior horn cells in Loa/+ mutants compared with wild type littermates, but there is no evidence of muscle denervation in Loa/+ mice, indicating that the pathology of the Loa mutation is a neuropathy.

A large intraspecific backcross between Loa/+ and the C57BL/6 inbred mouse strain was set up. Only affected N1 animals were backcrossed to C57BL/6 to generate more

than 1,000 N2 affected mice. The N2 progeny were used to map the Loa mutation to an interval of approximately 1.6 cM in the distal region of Mmu12. This interval is flanked by D12Mit17 and D12Mit181. We have constructed YAC/PAC/BAC contigs of the regions flanking the critical region and are generating more STS markers for chromosome walking and bridging the gap that exists within our contig of the Loa region. The STS markers are also analysed for simple sequence repeat and single nucleotide polymorphisms to narrow the critical region. The mouse and human comparative map, EST and other bioinformatics resources are routinely surveyed for identification of candidate genes near or within the critical region. Any candidate genes that map to the Loa critical region will be further analysed and if not excluded, they will be sequenced in affected and wild type mice for the identification of the Loa gene.

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J.P. Hatcher[1], P.T. Davey[1], S. Bingham[1], P. Overend[1], A.A Parsons[1], and J.B. Davis[1]. Vanilloid receptor-1 has a major role in thermal hyperalgesia.

The vanilloid receptor-1 (VR1) is a non-selective ligand-gated cation channel expressed in sensory neurones, which is suggested to have a major role in thermal nociception. Here we report studies in which we have investigated the responses of homozygous (-/-, n=10) and heterozygous (+/-, n=10) VR1 knockout mice , and their wildtype littermate controls (+/+, n=10) in the hotplate test and in the carrageenan model of thermal hyperalgesia. In the hotplate test there was a significant effect of genotype on withdrawal latency ($F=7.91$; df = 2,54; $P<0.001$). Follow up analysis showed no difference between -/- mice and +/+ controls at 50oC ($P=0.24$). At a hotplate temperature of 52.5oC there was a suggestion of an effect although this failed to reach significance ($P=0.09$). However at 55oC a significant difference was found ($P<0.01$). There was no significant difference between +/- mice and +/+ mice at any hotplate temperature. The same mice were then tested in the carrageenan model of thermal hyperalgesia, where analysis of variance showed a sigificant effect of genotype on latencies to withdrawal to a thermal stimulus post-carrageenan ($F = 5.54$; df = 2,52; $P<0.01$). Follow up analysis revealed that whilst both +/- and +/+ mice showed significantly decreased latencies post-carrageenan compared to baseline ($P<0.05$), -/- mice showed no such difference indicating no thermal hyperalgesia in these mice. These results confirm and extend the findings of Davis et al. (FENS Meeting; Brighton 2000) and show that VR1 receptors have a major role in the mediation of thermal hyperalgesia and that their role in thermal nociception is dependent on temperature.

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J.P. Hatcher[1], D.C. Rogers[1], C. Reavill[1], J.J. Hagan[1]. Use of SHIRPA to investigate the behavioural phenotype of the Coloboma (cm/-) mouse.

Coloboma mice have a 1.1 – 2.2 cM deletion on chromosome 2 which encompasses the Snap gene encoding the pre-synaptic nerve terminal protein, SNAP-25. The mutation is semi-dominant with the heterozygotes exhibiting a behavioural phenotype of spontaneous hyperactivity, constant head bobbing and a prominent eye dysmorphology (Hess and Wilson, 1992, J. Neurosci. 12: 2865-2874; Heyser et al., 1995, Dev. Brain Res. 89: 264-269). It has been suggested that such a mutation may be a model of Attention Deficit Hyperactivity Disorder (Hess et al., 1996, J. Neurosci. 16: 3104-3111). We have used the SHIRPA protocol (Rogers et al., 1997, Mamm. Genome 8: 711-713) to investigate the behavioural phenotype of these mice. Coloboma mice (cm/-) were found to have a number of differences in the primary observation screen when compared to wildtype controls including reductions in body weight and size, smaller eye openings and increased spontaneous activity. In a rotarod task, cm/- mice showed significantly shorter latencies than their wildtype littermates indicating that they may have motor co-ordination deficits. In a test of locomotor activity, cm/- mice showed a significant increase in activity over the 60 minute test period. This increase in activity was further enhanced upon a second exposure to the same apparatus. Our results indicate that cm/- mice show phenotypic differences from wildtype controls and may serve as a useful model for ADHD.

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A. Holmes[1], J.G. Hohmann[2], E. Yared[1], R.A. Steiner[2], and J.N. Crawley[1]. Variability in an anxiety-related phenotype in galanin over-expressing transgenic mice[3].

Galanin (GAL) is a 29 amino acid neuropeptide localized in brain regions relevant to the processing of emotional information, including hippocampus, amygdala, bed nucleus of the stria terminalis, hypothalamus, raphé nuclei, and locus coeruleus. Consistent with this expression pattern, intracranial administration of exogenous galanin produces increases and decreases in anxiety-related behaviors in rats, depending upon the site of administration and the behavioral test employed (Bing et al., 1993; Moller et al., 1999). To further explore the role of GAL in the mediation of anxiety-related behaviors, we generated transgenic mice overexpressing the GAL gene linked to a dopamine beta-hydroxylase promoter. GAL-tg mice are viable, and show normal neurological reflexes, motor, and sensory abilities. GAL-tg mice exhibit increased GAL mRNA in the locus coeruleus, elevated GAL peptide in the forebrain, and increased GAL fiber density in the hippocampus. Homozygous and heterozygous GAL-tg mice, and wild type littermate controls, were obtained for behavioral testing from the University of Washington in two separate batches. Heterozygotes and wild type littermates were bred at the Jackson laboratory (Bar Harbor, Maine). In experiment #1, female University of Washington GAL-tg mice displayed a heightened anxiety-like behavior in the light/dark transition test, but not in the elevated plus-maze test. In experiment #2, a second batch of female University of Washington GAL-tg mice displayed heightened anxiety-like behavior in both the light/dark transition test and the elevated plus-maze test. In experiment #3, female heterozygous GAL-tg mice bred at the Jackson laboratory showed reduced anxiety-like behavior in the elevated plus-maze, heightened anxiety-like behavior in the home base emergence test, and no significant phenotype in the light/dark transition test nor in novel object exploration. The variability in anxiety-related phenotype across separate batches of GAL-tg mice is discussed in relation to the effects of gene dosage, and also in terms of experimental variables, choice of test, and other factors common to studies with mutant mice.

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[3]Supported by NSF grant IBN-9720143 and the NIHM IRP.

T. Hough[1], P.M. Nolan[2], J. Peters[2], E.M.C. Fisher[3], J. Martin[4], M. Browne[5], S. Rastan[5], L. Vizor[2], S.D.M. Brown[2], and A.J. Hunter[1]. Clinical biochemistry screens can complement behavioural screens in mutagenised mice.

In the past phenotypic analysis of mutagenised, transgenic and knockout mice often focused solely on behavioural tests. In contrast the F1 offspring of mutagenised male mice from the SB/Harwell ENU programme are subjected to a routine blood biochemistry screen as part of a systematic phenotype-driven search for novel mouse mutations. The resulting data are used not only to identify possible biochemical mutants, but also to provide a complement to the behavioural screens. Consequently abnormal biochemical/physiological parameters can be ruled out as a precursor to behavioural anomalies. Following completion of all behavioural tests, at 8 to 12 weeks, around 300 ml of blood is collected from each F1 mouse. Samples are collected in Li-Hep capillary tubes from the tail vein and centrifuged at 3000 rpm for 10 minutes at 4°C. Approximately 125 ml of plasma is obtained. Kidney, liver, bone and lipid profiles, as well as glucose and bicarbonate tests are performed on an Olympus AU 400 analyser. Male and female data are analysed separately. Two criteria are used to identify potential outliers: mice with values > 3SD's from the running mean for any one parameter or >2SD's for groups of related parameters. Such potential outliers are re-tested after one month. In addition, offspring of mice identified from other components of the phenotype screen are also subjected to biochemical analysis. To date 1600 F1's have been screened and around 500 mice comprising 25 mutant lines (10 mutants, 10 controls per line) tested. Twelve F1 animals showing consistent abnormalities in plasma biochemistry were tested for inheritance of the mutation. Of these 12, 3 have currently been confirmed as inherited. Our results have shown that incorporation of such a biochemical screen is useful in characterising abnormal phenotypes/mutations. For example certain classes of behavioural mutants identified in this screen exhibited associated changes in biochemical parameters.

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Christopher Janus[1]. Behavioural mouse models of Alzheimer's Disease: A hit-or-miss approach?

The development of an animal model of memory impairment in Alzheimer's Disease (AD) is pivotal for understanding mechanisms of the disease and for exploration of new treatments. One of the main features of the disease is progressive cognitive and memory decline which coincides with amyloid plaques, neurofibrillary tangles, and neuronal loss. The heterogeneity of AD dementia presents a formidable challenge for the development of representative animal paradigms related to the cognitive impairment observed in AD. However, the potential advantages are enormous since the model will allow testing of biochemical phenotypes, dissection of essential pathological defects, and dissection of modifier genes and environmental factors. Difficulties in the development of the model are mainly imposed by species constraints of learning, hence careful choice of species and measured learning behaviour is crucial. Further, learning in animals most often is inferred from the analysis of their behavioural motor acts so impairments in the peripheral/sensorimotor systems may bias the analysis of cognitive systems.

With regard to commonly used laboratory animals, the development of transgenic (Tg) mice harbouring human genes implicated in AD established the feasibility of reproducing at least facets of the AD phenotype in a tractable ssystem. Also, the plethora of behavioural learning tests is most widely represented by a water maze test developed mainly for studying learning and memory in the rat, but is used as a routine basis for the analysis of mutant mice.

In my talk I focus on behavioural studies using Tg mouse models expressing familial AD mutations (PS1and APP genes) in the water maze test. Tg mice expressing mutant human PS1 (alleles M146L and L286V) did not have any detectable neuropathologic changes or show any noticeable sensorimotor or cognitive impairment in a conventional (place discrimination) version of the water maze test. However, naïve mutant PS1 mice showed initial impairment in the rate of acquisition of spatial information, both in simple cue learning and conventional place discrimination paradigms. Experienced Tg mice performed in a similar manner to controls, but when forced to acquire spatial information in a series of training sessions, the mutated Tg mice were consistently worse than Tg wild-type mice. In the case of $Tg(APP)_{CRND8}$ mice, spatial learning and memory was significantly impaired with respect to littermate controls, coinciding with the early onset of AD-related pathology in these mice. The $Tg(APP)_{CRND8}$ mice revealed yet a greater divergence from controls in the subsequent learning reversal tests, suggesting compromised behavioural flexibility.

I shall argue that more detailed analyses of mice behaviour in the water maze may be useful in characterisation of their cognitive impairments, and may convincingly demonstrate sensorimotor disturbances. Analysis of behavioural search strategies (thigmotaxis, search patterns) together with analysis in a cued (visible platform) version of the test may help us better understand group and individual variance. Exhaustive analysis of water maze mouse behaviour may first, lead to improved appreciation of compromised cognitive functions caused by AD transgenes, and second, may provide solid behavioural data for further experimentation which should ultimately yield a better understanding of AD-related impairment in humans.

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Christopher Janus[1], David Westaway[1], Jacqueline Pearson[1], Peter St. George-Hyslop[1]. Impaired spatial leaning and memory in APP CRND8 transgenic mice.

Dominant mutations in the ß-amyloid precursor protein (APP) are associated with familial Alzheimer's Disease (FAD). To address the relationship between FAD mutations and cognitive deficits, we created a novel line of Tg mice (TgCRND8) in a C3H/C57 genetic background, expressing a compound mutant form of the human APP695KM670/671NL+V717F. These mice exhibit very early-onset deposition of Aß-containing amyloid plaques, from 3 months onwards, with congophilic, neuritic plaques present from 5 months of age. The cognitive characteristics of the transgenic mice were compared to their non- Tg littermates. Since it is accepted that the hippocampal region is affected in the early stages of AD, we tested TgCRND8 mice in the hidden platform (place discrimination task) version of the Morris water maze (WM) at age coincident with the onset of AD-related pathology. We report that TgCRND8 mice showed a significant impairment in the acquisition of the spatial informa-

tion and, unlike non- transgenic littermates, did not develop a bias for the spatial position of the submerged platform. In the subsequent learning reversal test, the Tg mice were also significantly impaired in re-learning of the new spatial position and showed no spatial bias for the new position in the probe trial. During both learning tests Tg mice showed increased thigmotaxic swim behaviour, but in neither of the above tests did they differ significantly from non-Tg mice in their swim speed. We conclude that expression of mutated human APP in the TgCRND8 mice confers AD-related pathology at earlier times than reported for previous animal models of AD, and that profound impairments in spatial leaning and memory coincide with the onset of neuropathology. Our data suggest that TgCRND8 mice will be of great utility in assessing amyloid-directed therapies and the pathways linking Aß synthesis and cognitive impairment.

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Richard Lathe[1]. The enteroceptive hippocampus.

Hippocampal lesions produce deficits in specific types of learning, but its role in memory is unknown and the emphasis on memory processes may be misleading. To cast light on hippocampal function we examined the spectrum of genes expressed in the formation. Gene-trapping suggested that perhaps one third of the mouse genome is expressed in the hippocampus; the cases examined in detail all encoding membrane-associated signalling molecules (Steel et al., 1998, Hippocampus 8: 444-457). On further analysis the hippocampus was found to contain one of the highest densities of receptors for soluble ligands in the brain: these are accessible, functional, and mediate physiological and cognitive changes in vivo. The hippocampus is thus a primary target for ligands that reflect body physiology including ion balance and blood pressure, immunity, pain, reproductive status, satiety and stress. It is thus hypothesized that the early hippocampus diverged from the olfactory system (mediating exteroception), to sense soluble molecules in blood and cerebro-spinal fluid (enteroception).

It is suggested that the hippocampus may temporarily store this information via synaptic long-term potentiation (LTP): it is tempting to speculate that enhancement of LTP by 'good' ligands (e.g.s estrogen/fertility; alphaFGF/satiety) and impairment by 'bad' ligands (e.g., interleukins/infection; nociceptin/pain) is a relic of the early evolution of the hippocampus. If the hippocampus computes novelty by comparing sensory and memory inputs, as proposed by Vinogradova in 1975, then enteroceptive modulation provides that the hippocampus computes salience.

What then is the output of the hippocampus that reflects salience? Ablation and stimulation studies have demonstrated that the hippocampus acts as an endocrine transducer, and governs adaptive changes in body physiology and hypothalamic/pituitary/adrenal (HPA) axis activity. For instance, glucocorticoid levels rise in response to a novel stimulus, or to a previously learned aversive taste, the hormonal rise is abolished by hippocampectomy. Because adrenal hormones (glucocorticoids and norepinephrine) enhance consolidation of memory traces, endocrine activity directed by the hippocampus may explain its role in memory. In support, the requirement for the hippocampus in memory acquisition can be circumvented, at least in part, by co-administration of adrenal hormones. However, the hippocampus developed early in vertebrate evolution; more recent refinements may replace HPA axis modulation by neuronal relays that locally release norepinephrine in active regions of cortex.

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T. Lemberger[1], T. Mantamadiotis[1], O. Kretz[1], D. Gau[1], T. Steckler[2], and G. Schuetz[1]. Conditional mutagenesis of CREB in dopaminoceptive neurons.

Dopaminergic neurotransmission in basal ganglia and related pathways plays a major role in the regulation of movement, reward oriented behaviours, mnemonic functions like habit formation and in drug addiction. The CREB trancription factor is thought to be one important nuclear targets of the dopamine D1 receptor/cAMP/PKA signal transduction cascade. To analyze the contribution of CREB in this signaling pathway in vivo, we have conditionally disrupted the CREB gene in neurons expressing dopamine D1 receptors using the Cre/loxP system. For this purpose, we have generated transgenic lines using a 140 kb yeast artificial chromosome containing the dopamine D1 receptor gene to drive the expression of the Cre recombinase. The D1-Cre mice have been crossed with CREBlox mice in which exon 10 of the CREB gene is flanked by loxP sites. In the resulting "D1-CREB" (D1-Cre / CREBlox/lox) mutant animals the pattern of recombination, detected as a disappearance of the CREB protein, localizes to the re-

gions expressing Cre: striatum, nucleus accumbens, cortex layer VI, hippocampus CA2. Disruption of the CREB gene in the brain leads systematically to the upregulation of the CREM gene, a member of the ATF / CREB familly of transcription factors. To exclude that CREM can compensate for the loss of CREB, we have also generated double mutants D1-Cre / CREBlox/lox / CREMnull/null that should be completely devoid of any CREB-like activity in the D1 receptor expressing neurons. These mice are now being tested for striatum-dependent memory, response to psychostimulants and immediate-early gene induction.

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S. Lewis[1], M.A. Simoneau[1], S. Bailly[1], F. Guillou[1], B. Baron[1], and C. Belzung[1]. Behaviour of mice expressing human transferin in brain.

Transgenic mice expressing the human transferin gene in oligodendrocytes exhibit an important synthesise to myelin specific markers in the central nervous system in a B6D2 genetic background. We have compared behavioural characteristics of 3-month-old heterozygous female transgenic mice with their age-matched wildtype in five tests: Hugues box test for the measure of trait anxiety, light/dark box and elevated plus-maze for the measure of state anxiety, Porsolt test for depression symptoms and spatial open-field test for memory. No apparent motor deficit could be observed in all these tests. No difference was found between transgenics and wildtypes in anxiety tests. In Porsolt test, transgenic mice spent significantly less time escaping. In the spatial open-field, wildtype mice react to a change in the spatial configuration of objects by in increase of exploration, an effect that is not seen in transgenic mice, suggesting a deficit in spatial memory in these mice. These results show that mice expressing human transferin display specific changes of their behavioural pattern, which could be linked to a specific pattern of expression of myelin in the brain.

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Alicja L. Markowska[1], Alena Savonenko[1], and Katrin Andreasson[2,3]. Overexpression of cyclooxygenase-2 (cox-2) leads to cognitive impairment.[4]

Cognitive impairment in Alzheimer's disease (AD) is associated with inflammatory processes, which play a critical role in neuronal degeneration. This notion is consistent with findings from the recent studies suggesting that the use of non-steroidal anti-inflammatory agents, which inhibit the cyclooxygenase activity (cox-2), is protective against the development of AD (McGeer et al., 1996). In the present study we assessed the behavioral phenotype of an initial line of thy-1/hcox-2 transgenic mice, that overexpresses human cox-2. Our results indicate that the young thy-1/hcox-2 mice appear not to be different from age-matched non-transgenic littermates in spatial memory and in general phenotype such as activity, emotionality/anxiety, body balance and coordination, swimming ability, visual acuity, agility and sensorimotor reflexes. Once aged, these thy-1/hcox-2 mice showed accelerated impairment in spatial memory tasks dependent upon hippocampal function. This cognitive deficit coincided with the overexpression of the Cox-2 transgenic protein in the CA1 and CA3 regions of the hippocampus. These findings indicate that the overexpression of cox-2, which plays a role in the inflammatory processes may cause the deficit in cognitive function by itself or in interaction with aging.

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S.C. Maxson[1], A. Canastar[1], and C. Bishop[2]. Sex reversals (XX and XY females and XX and XY males), mating behaviors and aggressive behaviors in mice.

There are effects of one or more Y chromosomal genes on copulatory and attack behaviors of mice. These have been demonstrated for several pairs of Y chromosomal variants. It has been recently proposed that sex reversed mice might also be used to further investigate the effects of the Y chromosome on these behaviors. We tested XX and XY females on the C57BL6 and XX and XY males on the FVB background for copulatory and attack behaviors. Attack behavior was tested in a neutral cage with an opponent of the same genotype. Copulatory behavior was tested in the home cage with a female in hormonally induced estrus. The XX and XY females on the C57BL6 background did not differ in any of these behaviors, and it appears that the XX and XY males on the FVB background also do not differ on any of these behaviors. Several explanations will be discussed to possible account for the failure to find any differences.

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Peter McGuffin[1]. Heredity, hazards and the origins of depressive disorder.

Depression is a common disorder which, in addition to imposing a public health burden, imposes an economic burden in western industrialised countries that is rivalled only by cardiovascular disease. There is consistent evidence that depression is familial with the risk in the first degree relatives of sufferers ranging from three to nine times that in the general population. Recent population based and hospital register based twin studies show that familial aggregation is largely explained by genetic factors. That is, although the environment accounts for between 30% and 60% of the variation in liability, environmental factors appeared to be entirely of the non-shared type.

Older terminology suggested that depressive disorder could be sub-classified into "reactive" forms largely resulting from environmental insults and "endogenous forms" that were "constitutional", but there is little genetic evidence to support this and a recent twin-family analysis suggests that it is unlikely that there are two broad forms of depression, one mainly genetic and the other non-genetic. It is therefore probably that most cases of depression result from a combination of genetic liability and environmental adversity. However, the interplay between genes and environment appears to be complicated. Thus, there is evidence dating back to the mid-1980s that the relatives of depressed subjects not only show increased rates of depression, but also increased rates of experiencing (or reporting) threatening life events. The familial clustering of life events has been supported by twin studies which also have produced the surprising finding that life events are in some cases influenced by genes. This has led to the hypotheses that familial factors may influence the liability to depression indirectly by predisposing individuals to select a more "aversive" environment or, alternatively, there are some inherited cognitive schema that predisposes both to depressive symptomatology and reporting unpleasant happenings.

Meanwhile the molecular underpinnings of the genetic liability to depression remain obscure. Some promising leads have emerged from studies of candidate genes, particularly those involved in serotinergic transmission. While systematic whole genome scans have been undertaken in bipolar manic depression with controversial and conflicting results, linkage studies in unipolar depression taking a systematic approach have only just begun. Some of the approaches being embarked upon using linkage and linkage disequilibrium mapping will be described.

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K.L. McIlwain[1] and R.E. Paylor[1]. Effects of testing experience in a mouse behavioral test battery.[2]

In recent years there has been an increased interest in the behavioral phenotyping of genetically modified and in inbred strains of mice. Our laboratory uses a specific battery of tests for the initial assessment of phenotypic behavioral differences of transgenic and knockout mice, as well as inbred strains of mice**.** Our standard battery includes: open field activity, light-dark exploration, rotorod, prepulse inhibition (PPI), acoustic startle habituation, conditioned fear, Morris water maze, and hot plate. Mice are tested in the order listed, and this order for the test battery was devised from least invasive to most invasive, to decrease the chance that behavioral responses are altered based on prior test history. The studies presented here were designed around two basic questions. The first study addresses whether or not there are differences between mice that have undergone other previous testing and mice that are naïve to the test experience. The second study asks what is the effect of the testing order with respect to how an animal performs on subsequent tests? In the first experiment, one set of C57BL/6J male mice were evaluated on all of the tests described above. The behavior of these 'test battery' mice was compared to aged matched naïve mice that were only tested on one test from the battery. We found that on some tests the behavior of 'test battery' mice was significantly different from the behavior of naïve mice, while on other tests there were no differences between test battery and naïve mice. For example, test battery mice responded differently in the open-field, rotarod, and hotplate test, but behaved similar on the prepulse inhibition and fear conditioning test.

Experiments in the second study were carried out on male 129/SvEvTac and C57BL/6J male mice. In the second study we used an abbreviated battery of tasks, which included - open field activity, light-dark exploration, PPI, and fear conditioning. Given that it is not feasible to study all possible combinations, we chose 4 representative orders. Preliminary results from this second study suggest that certain test variables are sensitive to test order (such as latency to enter in the light-dark paradigm) whereas others (i.e. PPI) are resistant. These two studies demonstrate that some behavioral tests appear to be sensitive to previous testing experience, while other tests may be immune.

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Stéphane Mortaud[1], Laurent Nicolas[1], Isabelle Le Roy[1], and Pierre L. Roubertoux[1]. Attack behavior in mice: implication of the *sts* gene mapped on the pairing region of the X-Y chromosomes.[2]

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 pairing region of the Y co-segregates 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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Claudia F. Plappert[1], Peter K.D. Pilz[1], and H.-U. Schnitzler[1]. Sensitization of the acoustic startle response in mice differs between two inbred strains and is influenced by glycine.[2]

Sensitization is evoked by strong, aversive stimuli and causes a general increase in the strength of behavioural responses. The acoustic startle response (ASR) is a general coordinated muscle contraction elicited by loud acoustic stimuli which can be used to study two different sensitization paradigms. First, acoustic sensitization evoked by the startle stimuli themselves slows down or even prevents the normally observed habituation process. Second, electric sensitization evoked by footshocks increases the ASR compared to the response amplitude before footshocks. Acoustic sensitization and footshock sensitization interact if acoustic stimuli have a high SPL. High level acoustic stimuli produce strong sensitization preventing footshocks from eliciting additional sensitization.

We were interested in two questions: 1.) Is there a genetic influence on sensitization? If so, sensitization should differ between genetically different mouse strains. We examined two different strains, DBA/CN and BALB/CAN in both sensitization paradigms. 2.) Does glycine, a major inhibitory transmitter in spinal cord and brainstem, play a role in sensitization? If so, in spasmodic (spd) mutant mice with a defective glycine receptor alpha-subunit sensitization should be changed compared to the wildtype (WT).

For acoustic sensitization the mice were given 200 acoustic stimuli with either high SPL (25 dB above startle threshold) or low SPL (individually determined so that a weak ASR amplitude of about 100 mV was elicited in each animal). ASR amplitude was measured in a standard movement sensitive device. For electric sensitization, footshocks (0.5 mA, 500 ms duration, 1 shock/s) were presented between two series each consisting of 40 high SPL startle stimuli.

1.) High SPL stimuli produced higher ASR amplitudes in BALB than in DBA. The course of ASR during repetitve stimulation differed between the two strains. In BALB,

ASR initially increased and then only decreased slowly, while in DBA, ASR decreased about exponentially over the startle stimuli. Only with low SPL startle stimuli both strains showed about the same ASR amplitude and the same exponential amplitude decrease. We assume that in BALB sensitization by high SPL acoustic stimuli was very strong leading to high ASR amplitudes, and conteracting habituation, indicated by the initial ASR increase and the slow decrease afterwards. In DBA sensitization was only weak and habituation was able to succeed leading to a fast ASR decrease. With low SPL startle stimuli, that elicited the same weak ASR in both strains, the amount of habituation was the same indicating same weak sensitization in both strains in this case. Footshocks presented after high SPL startle stimuli elicited a much smaller ASR increase in BALB than in DBA. This confirms our hypothesis that in BALB acoustic sensitization is strong preventing additional footshock sensitization.

2.) In spd, high SPL startle stimuli elicited about 3-fold stronger ASR amplitudes as in the WT, and the ASR showed no time dependent changes. With low SPL startle stimuli, ASR in spd declined about exponentially over time, comparable to the WT in the high and the low SPL condition. Footshocks elicited no ASR increase in spd, but a strong increase in the WT. We assume that spd are at a high sensitization level producing high ASR amplitudes, counteracting habituation and preventing footshock sensitization.

Our results indicate that sensitization is genetically influenced. To locate the genes that are responsable for the stronger sensitization in BALB compared to DBA further genetic analysis (e.g. QTL analysis) of these strains will be necessary. Furthermore glycine plays a role in sensitization. We assume that a glycinergic tonic inhibition exits on the pathways mediating sensitization that is not described until now.

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Catharine H. Rankin[1], Jacqueline K. Rose[1], Kenneth Eng[1], and Karla Kaun[1]. Genetic dissection of habituation of the tap withdrawal response in *C. elegans.*[2]

Previous research has shown that the tap withdrawal response in the nematode C. elegans is composed of two competing reflexes that are integrated to produce the response. The tap withdrawal response shows short- and long-term habituation. The neural circuit underlying the response has been identified. The current research is designed to investigate the role of a number of specific genes expressed in identified neurons in the response to tap and in the plasticity expressed in the response. A number of strains of mutant worms were studied. The effect of the mutation on spontaneous behavior, on response to tap and on habituation to tap were tested. Several mutations affected spontaneous behavior without affecting the evoked behavior, while others altered habituation to tap without affecting the initial response to tap. Most of the strains tested were deficient in some aspect of glutamate transmission, with altered levels of presynaptic glutamate or altered post-synaptic receptors (AMPA-type, NMDA-type, GluCl-type or metabotropic type).

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C. Reavill[1], P. Nelson[2], J. Latcham[3], and J.J. Hagan[1]. Prepulse inhibition and startle responses in dishevelled (Dvl1-/-) mice.

Behavioural analyses of mice with selective chromosomal mutations are currently being conducted in order to link genes with disease states. Dishevelled (Dvl1-/-) mice have been reported to show reduced prepulse inhibition (PPI) (Lijam et al, 1997, Cell, 90: 895-905). Therefore we have studied PPI and startle responses in Dvl1-/- mice. Male and female mice were tested at 8 weeks of age in custom built startle chambers. For PPI trials, mice received either acoustic stimuli of white noise pulses (110dB/10 msec) or prepulses (4, 12 or 20 kHz/80 or 90 dB/10 msec) each followed 100 msec later by a white noise stimulus (110dB/10 msec). Dvl1-/- mice were further tested for PPI using a pulse stimulus of 100dB. In separate startle experiments mice received acoustic stimuli (4, 12, 20 kHz or white noise, 80, 90 or 110dB/10 msec). Repeated measures analysis of variance was used to analyse untransformed PPI responses and log transformed startle responses. There were no significant effects of genotype on PPI at a pulse of 110 dB ($F[1,34] = 0.22$; $P = 0.64$), 100 dB ($F[1,34] = 0.002$; $P = 0.97$) or on startle response ($F[1,34] = 0.43$; $P = 0.52$) compared to 129/SvEv littermates. Also, there was no significant effect of sex on PPI at a pulse of 110 dB ($F[1,34] = 0.21$; $P = 0.65$) or at 100 dB ($F[1,34] = 3.30$; $P = 0.08$). Contrary to previously published data (Lijam et al, 1997) Dvl1-/- mice did not show PPI deficits in this study.

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Michael Regulski[1], Grigori Enikolopov[2], and Tim Tully[1]. Genetics of NO signaling in the adult *Drosophila melanogaster* brain.

Nitric oxide (NO) is a transcellular messenger involved with a wide variety of physiological processes in vertebrates including vasodilation, immune response and synaptic plasticity. It has also been found to participate in developmental and behavioral plasticity in invertebrate nervous system including long-term memory formation in honeybees. We are interested in characterizing NO signaling in the fly adult brain. We have cloned and characterized dNOS gene, which codes for a Drosophila homolog of the nitric oxide synthase, the main source of NO. Immunocytochemical staining indicates that DNOS protein is expressed throughout the adult fly brain. We found that its expression levels rise for the first two days after eclosion and then decline. We think that these changes are linked to specific events in the final developmental stages of the fly brain. We carried out a screen for mutations in dNOS. Analysis of the phenotypes associated with these mutations will help us to understand NO functions in the fly adult brain.
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S.N. Schiffmann[1], G. Cheron[2], A. Lohof[3], P. d'Alcantara[1,5], M. Meyer[4], M. Parmentier[5], and S. Schurmans[5]. Calretinin, cerebellar network activity and motor coordination.[6]

The involvement of the cerebellum in motor control has long been recognized. However, the identification of this brain area as a primary site of motor learning is a largely supported but still controversial hypothesis. In the cerebellum, the parallel fiber-Purkinje cell synapse can undergo long-term synaptic plasticity suggested to underlie motor learning and resulting from variations in intracellular calcium concentration ([Ca^{2+}]i). Ca^{2+} binding proteins such as calretinin, calbindin and parvalbumin are enriched in the cerebellum but their role in information processing is poorly understood.

Mice deficient in calretinin ($Cr^{-/-}$) have been generated by gene targeting. They are impaired in tests of motor coordination such as the runway, the horizontal stationary rod and the wheel running test with a severe worsening with aging, suggesting functional deficits in cerebellar pathways. An impairment in Ca^{2+} homeostasis in Purkinje cells of $Cr^{-/-}$ mice was supported by the high Ca^{2+}-saturation of calbindin-D28K in these cells as demonstrated by the immunocytochemical and biochemical characterization of a paradoxical calretinin-like immunoreactivity.

The firing behavior of Purkinje cells analyzed in alert mice is severely affected in both 2-4 months- and 2-2.5 years-old $Cr^{-/-}$ mice with a 200% increase in the spontaneous simple spike firing rate, a 30-50% reduction in complex spike duration and a 70-85% reduction in the simple spike pause as compared with their wild-type littermates. In contrast, in cerebellar slices, excitatory synaptic transmission and short-term synaptic plasticity at parallel fiber- or climbing fiber-Purkinje cell synapses are unaltered, indicating that marked modifications of the firing behavior *in vivo* can be undetectable in slice.

These results show that calretinin plays a major role at the network level in cerebellar physiology without modification of properties of single cells examined so far and also show that the knock-out technique can unexpectedly « knock-in » new form of molecules as a neuron's reponse to these network changes.
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[6]Supported by PAI, FMRE and FRSM (Belgium) and Boerhinger-Ingelheim.

B. Schwaller[1]. The lack of parvalbumin affects the body and the mind.

Parvalbumin (PV) is a cytosolic low-molecular weight, high-affinity Ca^{2+}/Mg^{2+}-binding protein which is predominantly expressed in specific subpopulations of GABAergic interneurons in various brain regions. Additionally, PV is expressed in fast-twitch muscle fibres of rodents where the concentration is estimated to be in the millimolar range. To deduce possible functions of PV in these two tissues, PV-deficient mice (PV-/-) were generated by homologous recombination. In fast-twitch muscles of PV-/- mice the decay of $[Ca^{2+}]_i$ after 20 ms stimulation is slower compared to wild-type (WT) mice and leads to a prolongation of the time required to attain peak twitch tension and to an extension of the half-relaxation time. This is in good agreement with Ca^{2+}-measurements in PV-containing hippocampal neurones or PV-injected chromaffin cells. In both cases, PV as a slow-onset buffer -the rate of Ca^{2+}-binding being determined by the off-rate of Mg^{2+}- does not affect the amplitude of Ca^{2+}-transients, but

significantly increases the initial decay of $[Ca^{2+}]_i$ followed by a later exponential decay, thus even prolonging the transient. The knowledge about the metal-binding characteristics (affinities, kinetics) of PV is an absolute requirement for the understanding of the physiological role played in either muscle fibres or neurones.

The shape of cytosolic Ca^{2+}-transients in neurones are determined by the kinetics of Ca^{2+}-entry systems (channels in the plasma membrane or intracellular organelles), cytosolic Ca^{2+}-buffers such as PV or calbindin-D28k (CB) and finally by extrusion systems characterized by their Ca^{2+} extrusion rates. Simulation studies based on the experimental data from hippocampal neurones or PV-injected chromaffin cells indicate that slow buffers (PV) combined with high extrusion rates can mimic a very rapid Ca^{2+} extrusion mechanism.

One fundamental aspect of synaptic transmission, short-term plasticity, has long been known to be related to Ca^{2+} homeostasis, in particular to levels of $[Ca^{2+}]_i$ remaining after neuronal electrical activity ("residual Ca^{2+} hypothesis"). Interneurones from different brain regions (e.g. neocortex, cerebellum, hippocampus) contain high levels of PV, both in the soma and processes. PV was hypothesized to modify the time course of residual Ca^{2+} removal in terminals following an action potential, and hence modulate short-term plasticity. In the presentation, results from hippocampal and cerebellar electrophysiological recordings in PV-/- mice will be discussed. Furthermore, the lack of PV affects the spontaneous locomotor activity in these mice and is likely linked to the absence of PV in Purkinje cells. They contain the highest known concentrations of PV and CB compared to all other neurones. Mice deficient for both, PV and CB displayed a drastic decrease in locomotion, characterized by a reduced speed and a low percentage of fast movements. A recent finding on altered dendritic spine morphology of Purkinje cells in PV-CB double KO mice correlates well with the data on the impairment of spontaneous locomotor activity.

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Dennis A. Stephenson[1,2], Julie Gilchrist[1], Dionne Peterson[1], Sherry Tuner[1], Corike Nuibe[1], and George A. Carlson[1]. ENU induced behavioral mouse mutants which may involve APP or PrP.[3]

Induction of mutations that contribute to a phenotype (by enhancement or alleviating a characteristic) has proven to be highly effective in dissecting pathways that affect developmental processes in *Drosophila melanogaster.* Given this success with *Drosophila*, we have undertaken a mutagenesis programme to identify mutations at loci that might exasperate the mild phenotypic defects associated with 'knock-out' mutants at either the amyloid beta (A4) precursor protein or prion protein locus. Offspring from ENU (ethyl nitrosourea) treated male mice were subjected to a serious of simple behavioral and observational screens. To date, we have screened in excess of 1500 G1 progeny and observed sixteen heritable mutations. Three involved mutations at the tyrosinase (albino) locus providing an estimate of mutation frequency consistent with published data. Several exhibited gait abnormalities while other displayed reduced body weight. Two abnormal gait mutants (lines 67 and 81) exhibit phenotypic variations that appear to depend upon the presence or absence of either App or Prnp gene. Line 67 exhibit episodes of slow deliberate movement which last for a few days around weaning with a mild residual high stepping gait which persists into adult life. Other characteristics include plastic tail, 'praying behavior' and poor co-ordination that are also episodic. Presumptive mutant homozygotes die before weaning and exhibit a more severe phenotype with earlier onset. The most prominent characteristic associated with these presumptive homozygotes is a difficulty to right themselves when they roll on their back. Line 81 has a more complex phenotype however, the most persistent characteristic is an intention tremor. PrP-null homozygotes are significantly more reactive to changes in environmental conditions (e.g., hyperactive, aggressive) than null heterozygotes. Detailed descriptions of both mutants will be presented along with video recordings in an attempt to get a better understanding of their clinical significance.

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Fred van Leuven[1]. Transgenic mice and Alzheimer pathology: no need for plaques or tangles?

We have generated different strains of transgenic mice that overexpress either wild-type or mutant Amyloid Precursor Protein (APP), human wild-type or mutant Presenilins (PS1, PS2), human ApoE4 or human protein tau. All constructs were based on the mouse thy1 gene promoter to express the transgenes specifically in neurons and all mice were gen-

erated and maintained in the FVB background.

Remarkably, all APP transgenic mouse strains displayed in essence a similar behavioral phenotype as the original APP/RK mice (Moechars et al., 1996, EMBO J. 15:1265-1274; 1999, J. Biol. Chem. 274:6483-6492). The major symptoms include: disturbed behavior of reduced exploration, neophobia, increased aggression, excitotoxicity with premature death and hypersensitivity to kainic acid, but hypo-sensitivity to NMDA with reduced cognition and defective LTP. The differences between the APP transgenic strains were quantitative in nature, i.e. differences in intensity and in severity, or differing in the age of onset of the symptoms. These quantitative differences correlated with the level of expression of the transgene and with its nature, i.e. wild-type APP less severe than mutant APP. All the symptoms mentioned are "early defects", i.e. obvious at ages ranging from 3 and 9 months. Measured in the Morris watermaze, the cognitive deficit was most marked in APP/london transgenic mice even when only 3 to 6 months, but was also observed in APP/wt transgenic mice (Moechars et al., 1999, J. Biol. Chem. 274:6483-6492).

The common early symptoms are in obvious contrast with the absence of amyloid plaques in the APP/RK and APP/wt mice and with the late appearance of amyloid plaques together with vascular amyloid in the brain of the APP/london transgenic mice. These lesions developed only in the highest expressing APP/london transgenic strain, and only when these mice were 12 months or older. The biochemical correlate of the phenotype and of the occurrence of plaque and vascular amyloid became evident from analysis of the brain levels of membrane-bound APP, of α- and ß- secreted APP, of the C-terminal "stubs" (C99) and of Aß40 and Aß42. No single intermediate correlated completely with the complex of phenotypic dysfunctions. On the other hand, the development of amyloid plaques in the APP/london mice was direct correlated to high Aß42 levels. The histochemical and immunochemical characteristics of the amyloid plaques and of the vascular amyloid recapitulate closely the amyloid pathology of AD patients (Van Dorpe et al., 2000, Am. J. Pathol. in press). This includes immunoreactivity for hyper-phosphorylated tau in swollen neurites around the amyloid plaques, but without the neurofibrillary inclusions of tau-pathology seen in AD patients.

The combined observations in the APP transgenic mouse strains demonstrate above all the marked dissociation in time of the early cognitive and behavioral deficits observed in all APP transgenic strains that we have generated and characterized, from the late and selective development of amyloid plaques only in old APP/london mice that produce high levels of Aß42. Whereas the occurrence of plaque and vascular amyloid is explained by these higher levels of Aß42, the cognitive and behavioral phenotypic traits must be linked to other metabolites of APP, i.e. Aß40, ß-C-stubs and secreted APP, most likely in combination. To define their respective contributions, other and more complex transgenic mouse strains are being generated.

Double transgenic mice, i.e. APP/london x PS1[A246E], develop amyloid plaques when only 6-9 months old, concomitant with increased Aß42 levels. The other APP-metabolites are relatively unchanged, which is concordant with the observation that the early behavioral traits in the APP/Lo x PS1 double tg mice are not essentially different from the single APP/london tg mice. Single PS1 tg mice that overexpress either the wild-type human PS1 or the EOFAD mutant PS1[A246E], have essentially no pathology or phenotypic abnormalities. Mice deficient in PS1 are not viable, but primary cultures of embryonal neurons grow and differentiate normally, and were used to demonstrate that production of the amyloid peptides is reduced dramatically in the absence of PS-1 (De Strooper et al., 1998, Nature 391:387-390).

To overcome the lethality of the PS1 deficiency, we have now generated mice that are neuronally deficient in PS1: mice with a "floxed" PS1 gene were crossed with transgenic mice that overexpress cre-recombinase in neurons by way of the thy1 gene promoter. The viable offspring accumulate ß-C-stubs in their brain from endogenous mouse APP, demonstrating in vivo the metabolic impact of PS1 deficiency on γ-secretase cleavage (Ilse Dewachter et al., unpublished results). These mice are being characterized and further crossed with APP/london transgenic mice to yield "triple" transgenic mice, i.e. homozygous for floxed PS1, and heterozygous for thy1-Cre-recombinase and for thy1-APP/London. In essence these mice overexpress human APP/london in the same neurons that are deficient in PS1 and will be instrumental to answer the question if and how accumulated ß-C-stubs are pathological or beneficial. This constitutes the in vivo paradigm of the therapeutic intervention in AD patients aimed at inhibition of γ-secretase to reduce production of the amyloid peptides which would entail accumulation of their obligate immediate precursors, the ß-C-stubs of APP.

Although the APP/london mice faithfully recapitulate the plaque and vascular amyloid pathology of AD patients and also demonstrate interesting cognitive and behavioral problems, the pathological aspect of neurofibrillary tangle formation is lacking. This is a paradox and could also explain the lack of an early cholinergic deficit in the APP/london mice, whereby the cholinergic defect is restricted to disturbed cholinergic tracts associated with amyloid plaques in old APP/London mice.

To implement and understand the problem of tau-pathology in AD, we have generated transgenic mice that overexpress human protein tau and two suspected tau-kinases, GSK-3ß and cdk5 with its activator p35. Overexpression of protein tau4R in neurons results in transgenic mice that were psychomotorically impaired and developed prominent axonopathy in brain and spinal cord. Axonal dilations with accumulation of neurofilaments, mitochondria and vesicles are prominent suggesting that defective axonal transport causes axonal degeneration. This effect was genedosage related, it proved that merely increasing the concentration of the four-repeat tau protein isoform is sufficient to cause neuronal injury without additional requirement of intraneuronal neurofibrillary tangles (Spittaels et al., 1999, Am. J. Pathol. 155: 2153-2165).

Since patients with AD or other tauopathy develop tau inclusions that consist of hyper- phosphorylated protein tau, tau kinases are thought to be actively involved. Overexpression of GSK-3β in neurons of single and double transgenic mice, provided evidence that GSK-3β is indeed an effective protein tau-kinase in vivo. Hyperphosphorylation of murine and human protein tau was exemplified by the appearance of isoforms with slower electrophoretic mobility, immunoreactive with monoclonal antibodies AT-8 and AT-180, among others that are certified to recognize typical phosphorylated tau epitopes in AD brain. Further analysis at the ultrastructural level will reveal whether and how tau phosphorylation is involved in tangle formation or in the other neuro-degenerative processes.

ApoE4 is an important genetic risk-factor for AD, but besides the epidemiological evidence, the molecular contribution of ApoE4 to the neurodegenerative pathogenesis is not known. Rodent neurons do not express any ApoE, as opposed to astrocytes, while some evidence is available that human brain regions in which neurons express ApoE might be most vulnerable for developing neurofibrillary pathology. We tested the hypothesis that the expression pattern of human ApoE is important or even deciding for the pathogenesis of AD in carriers of ApoE4 alleles. We have generated transgenic mice that over-express human ApoE4 in either neurons (thy1 gene promoter) or astrocytes (GFAP gene promoter).

Transgenic mice with neuronal expression of human ApoE4 progressively exhibited motoric problems that correlated with neuronal hyper-phosphorylation of protein tau. Neurons in brain and spinal cord reacted positively with monoclonal antibodies AT8, AT180 and PHF1, which specify AD related epitopes. Increased protein Tau phosphorylation was dependent on the level of neuronal expression of human ApoE4 and on the age of the mice. In addition, ApoE4 transgenic mice developed axonopathy, severe motor impairment and neurogenic muscle atrophy (Tesseur et al., 2000, Am J Pathol, 156, 3, 951-964, Am. J. Pathol. in press). Numerous inclusions stained positive for ubiquitin, neurofilaments and synaptophysin in the white matter tracts of the CNS, indicating impairment of axonal transport. This was confirmed at the ultrastructural level and was similar but not identical to defects in our transgenic mice that overexpress human protein tau. In sharp contrast, none of these symptoms were detected in transgenic mouse lines that over-express human ApoE4 in astrocytes at similar levels. Our data link, for the first time, the genetic risk-factor ApoE4 to a pathological defect in AD, i.e. hyperphosphorylation of protein tau. The polymorphisms in the ApoE gene promoter that constitute increased risk for developing AD could then also cause or define expression of ApoE in neurons, and, combined with our results, offer a mechanism for the pathogenic role of the ApoE4 allele in AD.

Experiments in "multiple" transgenic mice are ongoing and other relevant genes are being implemented to determine which of the APP metabolites is causing the early signs of the "amyloid"-related phenotype, how "tau-pathology" is related and to be implemented and how the neuronal "ApoE4-tau" connection is operating, to eventually determine the importance, if any, of the intraneuronal tangles, the other lesion essential for diagnostics of AD.

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Fred van Leuven[1]. Transgenic mouse models for Alzheimer's disease.

ApoE4 is an important genetic risk-factor for AD, but besides the epidemiological evidence, the molecular contribution of ApoE4

to the neurodegenerative pathogenesis is not known. Rodent neurons do not express ApoE, as opposed to astrocytes, while some evidence is available that human brain regions in which neurons express ApoE might be most vulnerable for developing neurofibrillary pathology. We tested the hypothesis that the expression pattern of human ApoE is important or even deciding for the pathogenesis of AD in carriers of ApoE4 alleles. We have generated transgenic mice that over-express human ApoE4 in either neurons (thy1 gene promoter) or astrocytes (GFAP gene promoter). Transgenic mice with neuronal expression of human ApoE4 progressively exhibited motoric problems that correlated with neuronal hyperphosphorylation of protein tau. Neurons in brain and spinal cord reacted positively with monoclonal antibodies AT8, AT180 and PHF1, which specify AD related epitopes. Increased protein Tau phosphorylation was dependent on the level of neuronal expression of human ApoE4 and on the age of the mice. In addition, ApoE4 transgenic mice developed axonopathy, severe motor impairment and neurogenic muscle atrophy (Tesseur et al, Am J Pathol, 2000, 156: 951-964). Numerous inclusions stained positive for ubiquitin, neurofilaments and synaptophysin in the white matter tracts of the CNS, indicating impairment of axonal transport. This was confirmed at the ultrastructural level and was similar but not identical to defects in our transgenic mice that overexpress human protein tau. In sharp contrast, none of these symptoms were detected in transgenic mouse lines that over-express human ApoE4 in astrocytes at similar levels. Our data link, for the first time, the genetic risk-factor ApoE4 to a pathological defect in AD, i.e. hyper-phosphorylation of protein tau. The polymorphisms in the ApoE gene promoter that constitute increased risk for developing AD could then also cause or define expression of ApoE in neurons, and, combined with our results, offer a mechanism for the pathogenic role of the ApoE4 allele in AD.

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A.H. Veenema[1], G.A. Van Oortmerssen[1], A.J.H. De Ruiter[1], B. Bohus[1], J.M. Koolhaas[1], and F Sluyter[2]. Neurobehavioral effects of the Y chromosome in wild house mice.

In mice artificial selection is one of the most powerful tools to demonstrate the contribution of genetic variation to individual differences in aggressive behavior. In Haren, The Netherlands, selective breeding in wild house mice has resulted in two lines: [1] a fast attacking, aggressive line characterized by short attack latencies (SAL) and [2] a slow attacking, non-aggressive line with long attack latencies (LAL). However, aggression is not the only behavior in which these lines vary and it is generally believed that they differ more fundamentally with each line showing its particular neurobehavioral characteristics. An important genetic candidate that might explain this neurobehavioral variation is the Y chromosome. To investigate Y chromosomal effects, congenic lines have been developed (named SAL.LY, LAL.SY), which only differ from their parental lines (SAL, LAL) with regard to the Y chromosome (i.e. the nonpairing part). Comparisons between SAL.LY and SAL on the one side and LAL.SY and LAL on the other side may then reveal possible neurobehavioral effects of the Y chromosome. This presentation reviews the results of previous studies of Y chromosomal effects on behavior (aggression, defensive burying), neurochemistry (apomorphine-induced stereotypy) and neuroanatomy (the sizes of the hippocampal inter- and intrapyramidal mossy fibers terminal fields) in these selection lines. Moreover, it investigates whether Y chromosomal variation affects the behavior of these mice in four paradigms suggested to study to some extent anxiety and/or depression levels: the elevated plus maze, open field, sudden silence test and Porsolt's swim test.

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S. Viñas[1], S. Lewis[1], S. Barreau[1], C. Ducottet[1], A. Aubert[1], and C. Belzung[1]. Strain differences in two animal models of depression : the forced swimming test and the chronic mild stress procedure.

The present study was aimed at investigating strains differences in animal models of depression. We focused on two models: the forced swimming test and a chronic mild stress procedure. The effects of chronic mild stress were observed on sexual behavior, sucrose consumption and fur state of the mice (cleaned or not). Mice from 9 different inbred strains were used: BALB/cByJ, C57BL/6J, 129Sv/ter, AKR/OlaHsd, CBA/J, C3H/HeNHsd, AJOlaHsd, SJL/JHanHsd and DBA/2OlaHsd.

Results show: 1) in the forced swimming test, immobility duration was the highest in C57, AKR and 129 mice while swim dura-

tion was higher in BALB and DBA mice; 2) in the chronic mild stress procedure, the main effect was seen on fur state. Indeed, this state remained optimal during the 4 weeks of stress in C57 mice while it worsened in BALB and DBA mice after 1 week and in AJ and SJL after 3 weeks of stress.

We conclude that the choice of a given strain is an important factor, that should be considered when studying the effects of a given pharmacological treatment or a given mutation. Moreover, present results show that the two animal models of depression may not measure the same psychological state, because they are not sensitive to the same factors of variation.

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A.L. Vyssotski[1,2], G. Dell'Omo[1,3], D.L. Vyssotski[2], D.P. Wolfer[1], L. Minichiello[4], R. Klein[4], I.I. Poletaeva[5], and H.-P.Lipp[1]. Mice lacking the neurotrophin receptor TrkB in the forebrain show intact spatial memory but impaired behavioral flexibility in a semi-naturalistic set-up.[6]

Mice lacking the neurotrophin receptor TrkB in the forebrain have been found to be unable to learn the Morris water maze task, to be impaired in radial maze learning, while they appeared less or not affected in more simple learning tasks such contextual fear conditioning (Neuron 24, 401-414, 1999). In order to clarify how such an impairment would affect the learning ability in a natural environment, we have developed a system consisting of eight computer-controlled feeder/trap units. This set-up permits to deliver (or withhold) food reward to individual transponder tagged mice visiting the traps for obtaining their daily food. The system operates inside a mouse colony kept permanently outdoors (or indoors), and records continuously visits of every mouse. After an individual mouse has entered a feeder box, other mice are barred from entering until the occupant has left. Thus, the following variables are recorded for each individual: i) time of visit; ii) place of visit; iii) food reward or not (correct choice and later reentries). The task resembles a radial maze test but is more complicated because of protracted food delivery schedules and the necessity of adjusting behavior when faced with a box occupied by another mouse feeding inside. On the other hand, it emulates the everyday learning requirements of mice quite nicely.

Forty mice (11 wildtypes, WW; 21 heterozygous, WM; and 8 mutants, MM) were released into an oudoor pen of 10x10 m. Four feeder boxes were placed in distant corners, four boxes in proximity to two central shelters offering protection against wind and rain. Predators were barred from the pen by nets and electrical fences. A daily feeding cycle started at 8 p.m. and lasted overnight till 8 a.m. During this time, a mouse would receive a food reward of about 0.5 g at every box but only during the first visit. As it was not certain whether poorly learning mice would receive enough food, additional free food was placed inside the shelters every third day.

It was found that all 3 groups (WW, WM and MM) gradually learned to visit all eight feeders during a night, without significant differences between the groups. This indicates that the TrkB mutants did not suffer from spatial memory deficits nor from basic learning inabilities. However, differences between mutants and wildtypes emerged gradually at those days with free food inside the shelters. While the wildtypes soon abandoned to visit the outside feeders during such nights, the TrkB mutants continued to patrol the boxes in their habitual way. For example, on day 21, the average number of boxes visited by the mutants was 7.0, while the average visits to those boxes dropped to 0.86 in the wildtypes (Mann-Whitney, $p<0.007$). The heterozygous animals performed in-between (average visits 4.7, different from wildtypes, Mann-Whitney, $p< 0.002$).

In conclusion, these data show rather convincingly that TrkB deficient mice were able to learn but, once having learned a task, were almost unable to switch quickly to another behavioral strategy. This implies that the TrkB neurotrophin receptor must play a fundamental role in behavioral flexibility. In addition, natural learning set-ups monitoring a collective of mice permit to recognize such slowly emerging behavioral changes (or lack thereof) much better than single test episodes of individual mice.

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David Westaway[1]. Transgenic mouse models of Alzheimer's Disease: long-haul or home-stretch?

Although there is intense interest in recreating an accurate murine model of Alzheimer Disease (AD), there are considerable obstacles associated with this goal. It has been suggested that accurate models should fulfill some simple expectations including: 1) Progressive neuropathology culminating in several accepted pathologic hallmarks of AD (plaques, tangles, loss of cholinergic neurons). 2) Presence of cognitive deficits as well as neuropathologic changes noted in 1. Such cognitive changes should be robust, and evident in different behavioural paradigms targeting the same memory system. Ideally, behavioural paradigms employed to test mice should address neuroanatomic structures that are affected in AD (e.g., the hippocampus). 3) In the case of experiments employing familial Alzheimer Disease (FAD) mutations, phenotypic changes documented in 2 and 3 should be correlated with the presence of the FAD mutations, and should be absent or less overt in mice expressing wt gene alleles expressed at equal (or greater) steady-state levels. 4) Recapitulation of phenotypic changes as per items 1-3 in independent Tg lines harbouring the same construct, to exclude the contribution of insertional mutations or linked loci. 5) Confirmation of key phenotypic traits in independent laboratories.

As there are few AD models addressing these criteria, this laboratory has also entered the fray to create transgenic (Tg) mice with AD-related pathology. Using a mutant form of APP695 inserted into the cos.Tet prion promoter expression vector, microinjections were carried out into a mouse genetic background partially protective against the effects of APP over-expression. This strategy allowed the establishment of a Tg line expressing high levels of mutant APP and designated TgCRND8. Aβ is readily detectable in the brains of TgCRND8 mice by western blotting, from 120 days onwards. Fields of amyloid plaques can be detected in the cortex and hippocampus by immunohistochemistry from 90 days of age. Congophilic and birefringent Aβ deposits with similarities to senile plaques of AD are seen from an early age, but although dystrophic neurons are present, neurofibrillary tangles have yet to be detected. Co-expression of either mutant PS1 or PS2 greatly accelerates amyloid deposition in TgCRND8 mice, to the extent that dense fields of plaques are evident from between 43 to 90 days of age, depending upon the particular presenilin mutation. TgCRND8 mice respond to immunization with Aβ42, although there are quantitative and qualitative differences from studies reported by Schenk and co-workers in PDAPP mice. The advantages and shortfalls of TgCRND8 mice as (i) a credible AD model as per the criteria described above and (ii) a vehicle to decipher the impact of amyloid deposition and amyloid-directed therapeutics on neuropathology and cognitive function will be discussed.

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