For example, could the auditory cortex recover after the 'retardation' and eventually function as it does in normal adults? What are the cellular and molecular bases that would allow the putative reopening or prolongation of the critical period? The end of the critical period in cortical development of normal animals has been shown to correlate with the changes in certain neurotransmitter receptor properties (e.g. NMDA receptors) as well as with the level of brain-derived neurotrophic factor (BDNF) [19–21]. It would be interesting to know whether behavioral manipulations used by Chang and Merzenich altered underlying molecular signaling mechanisms in auditory cortex. Studies of these questions will help us understand whether the extended cortical plasticity observed in noise-reared animals is an extension of the critical period that is observed in normally raised animals or whether it constitutes another form of cortical plasticity.

Nevertheless, the notion of a 'plastic critical period' has tremendous implications. Studies along this line could uncover unified mechanisms that link all types of cortical plasticity over the lifespan of an animal or human. Such findings should also have therapeutic implications for treating children with hearing loss at young ages. If the outcome of the Chang and Merzenich study and of studies of electrical stimulation in congenitally deaf cats [11] are of any indication, methods to help regain normal function of auditory cortex, and consequently speech and language processing ability, in effected children are some of the promising possibilities just over the horizon.

References
1 Hubel, D.H. and Wiesel, T.N. (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J. Physiol.* 206, 419–436
2 Moore, D.R. (1985) Postnatal development of the mammalian central auditory system and the neural consequences of auditory deprivation. *Acta Otolaryngol. Suppl.* 421, 19–30
3 Zhang, L.I. *et al.* (2001) Persistent and specific influences of early acoustic environments on primary auditory cortex. *Nat. Neurosci.* 4, 1123–1130
4 Zhang, L.I. *et al.* (2002) Disruption of primary auditory cortex by synchronous auditory inputs during a critical period. *Proc. Natl. Acad. Sci. U. S. A.* 99, 2309–2314
5 Chang, E.F. and Merzenich, M.M. (2003) Environmental noise retards auditory cortical development. *Science* 300, 498–502
6 Irvine, D.R. *et al.* (2001) Injury- and use-related plasticity in adult auditory cortex. *Audiol. Neurootol.* 6, 192–195
7 King, A.J. and Moore, D.R. (1991) Plasticity of auditory maps in the brain. *Trends Neurosci.* 14, 31–37
8 Recanzone, G.H. *et al.* (1993) Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J. Neurosci.* 13, 87–103
9 Kral, A. *et al.* (2002) Hearing after congenital deafness: central auditory plasticity and sensory deprivation. *Cereb. Cortex* 12, 797–807
10 Kral, A. *et al.* (2001) Delayed maturation and sensitive periods in the auditory cortex. *Audiol. Neurootol.* 6, 346–362
11 Klinke, R. *et al.* (1999) Recruitment of the auditory cortex in congenitally deaf cats by long-term cochlear electrostimulation. *Science* 285, 1729–1733
12 Wiesel, T.N. (1982) Postnatal development of the visual cortex and the influence of environment. *Nature* 299, 583–591
13 Merzenich, M.M. *et al.* (1983) Progression of change following median nerve section in the cortical representation of the hand in areas 3b and 1 in adult owl and squirrel monkeys. *Neuroscience* 10, 639–665
14 Merzenich, M.M. *et al.* (1984) Somatosensory cortical map changes following digit amputation in adult monkeys. *J. Comp. Neurol.* 224, 591–605
15 Kaas, J.H. *et al.* (1990) Reorganization of retinotopic cortical maps in adult mammals after lesions of the retina. *Science* 248, 229–231
16 Pons, T.P. *et al.* (1991) Massive cortical reorganization after sensory deafferentation in adult macaques. *Science* 252, 1857–1860
17 Cynader, M. and Mitchell, D.E. (1980) Prolonged sensitivity to monocular deprivation in dark-reared cats. *J. Neurophysiol.* 43, 1026–1040
18 Mower, G.D. (1991) The effect of dark rearing on the time course of the critical period in cat visual cortex. *Brain Res. Dev. Brain Res.* 58, 151–158
19 Katz, L.C. (1999) What's critical for the critical period in visual cortex? *Cell* 99, 673–676
20 Berardi, N. *et al.* (2003) Molecular basis of plasticity in the visual cortex. *Trends Neurosci.* 26, 369–378
21 Bear, M.F. and Rittenhouse, C.D. (1999) Molecular basis for induction of ocular dominance plasticity. *J. Neurobiol.* 41, 83–91

Dissecting complex behaviours in the post-genomic era

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Identifying gene functions in behaviours has so far relied mainly on achievements in the field of molecular genetics. Further progress can be made by developing new approaches that allow refinement of behavioural phenotypes. The current availability of several thousand different mutant mice challenges behavioural neuroscientists to extend their views and methodologies, to dissect complex behaviours into behavioural phenotypes, and subsequently to define gene–behavioural

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phenotype relationships. Here, we plead for multi-day automated behavioural observations in carefully designed environments.

Behavioural testing in animals, a crucial feature of phenotyping in neuroscience, is usually based on measuring behavioural responses to environmental events that are induced by the experimenter. The frequently used open-field test, for example, is commonly employed to study general activity and fear-related behaviours in mice and rats [1,2]. In this test, movements of the animal are monitored up to one hour after the animal has been placed in a novel open arena from which it cannot escape. Additional tests, such as the elevated plus maze [3] and the light−dark box [4], allow external validity of the observed open-field behaviours. However, these tests are short-lasting, and depend on individual locomotor activity levels and novelty responsiveness of the animal, as well as on human interference. This hampers their use for determining gene−behavioural phenotype relationships and stresses the need for new analytical procedures addressing the complex behaviours. Although some ideas for overcoming these problems have been put forward, such as improving currently available tests, using test batteries and increasing test information density [5,6], behavioural complexity and gene−environment interactions require new methodology in this field of research.

## Interacting physiological processes

Expression of a particular behaviour at a certain time is triggered by the integration of internal and external signals (such as hunger and food availability, respectively) and is guided by the ability of the animal to execute proper behavioural responses. For instance, a hungry mouse that searches for new food resources relies on an efficient exploration strategy in which finding the food resource in time and taking the risk of being exposed to predators need to be balanced. Furthermore, in the wild, mice face the risk of using more energy to gather food than the obtained food gives them in return on any given day. Because exploration for food is influenced by different integrated physiological processes (e.g. energy balance, motor action and fear), as well as by environmental factors (e.g. variations in ambient temperature, in food availability and in photoperiod), the design of behavioural laboratory methods that dissociate the various behavioural components is a challenge. Although limitations in behavioural testing environments and duration are practical realities of the mouse laboratory, these parameters should be carefully chosen and directed by a clearly defined working hypothesis.

Conventional laboratory tests, such as the open-field arena, touch upon different aspects of exploratory behaviour, such as locomotor activity and fear-related processes. However, during the relative short testing episode generally employed, it is impossible to discriminate between gene function in novelty-induced and baseline behaviours. For example, mice that lack the dopamine transporter gene have behavioural locomotor activity levels under baseline conditions that are comparable to those in wild-type animals, but they exhibit a > 12-fold increase of locomotion following placement in a novel environment. Although dissociation of novelty-induced and baseline locomotor behaviour in this mutant was observed during a relative short-lasting testing procedure, the time of day that these tests are performed can highly influence the outcome of the observations [7−9] (Figure 1). Thus, characterization of gene functions in exploratory behaviour requires behavioural paradigms that allow dissection of this complex behaviour into different components in view of circadian-induced variations of these components.

Interference and order effects

Executing multiple behavioural tests usually involves experimenter interference, such as handling or transport
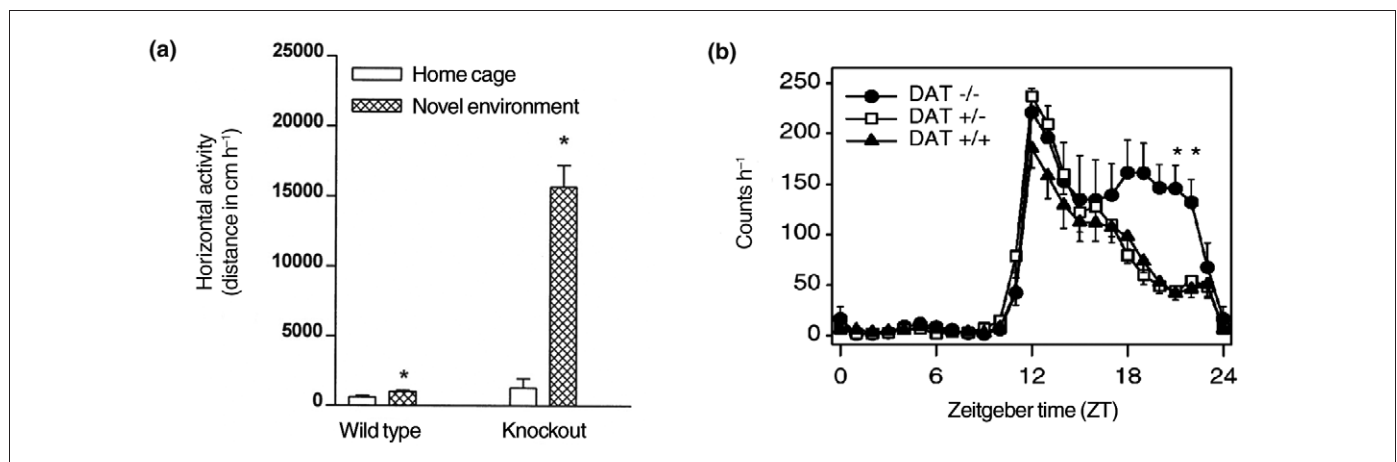
Figure 1. Dissociation of gene function in behavioural regulation requires assessment of novelty-induced and baseline behaviours during the appropriate circadian phase. Although brief assessments of behaviour (e.g. for 1 h) are attractive with respect to the screening capacity, they can yield discrepancies with longer assessments of behavioural testing (e.g. 24 h). For example, hyperactivity in mice that lack the gene encoding the plasma membrane dopamine transporter (DAT) appeared to be novelty driven because locomotor activity was ∼12-fold higher in an environment that was novel to the mouse at the start of the experiment (a). Asterisks indicates $P < 0.05$ for activity in familiar versus novel environments. No significant differences between the activities of DAT-knockout and wild-type mice were observed in their home cages during the 1 h of behavioural monitoring. (b) In another study, 24-h rhythms in behavioural activity were measured in DAT $-/-$, DAT $+/-$ and wild-type (DAT $+/+$) mice. Locomotor activity levels were higher in DAT $-/-$ animals, but only during the last part of the dark phase while housed in home cages. Thus, continuously behavioural observations can be crucial for gene function characterization in novelty-induced and baseline behaviours that can vary across different phases of the 24-h cycle. Zeitgeber time (ZT) 0−12 and ZT 12−24 refer to the 12-h light and the 12-h dark phases of the 24-h light−dark cycle, respectively. Panel (a) reproduced, with permission, from Ref. [8] © (1999) American Association for the Advancement of Science; (b) reproduced, with permission, from Ref. [7] © 2001 by the Society for Neuroscience.

of animals [10], and cues from the experimenter that influences the behavioural performance of an animal [11] (Figure 2). For example, measuring pain responses in mice revealed that experimenter effects account for more trait variability than genotype [12]. In addition, recent studies have shown that simultaneous testing of several inbred mice strains and a mutant mouse strain across three laboratories resulted in behavioural expression differences, despite standardization of the test procedures [13]. Mice exposed to a battery of various behavioural tests expressed significant lower levels of locomotor activity in the open field than mice that were naïve to behavioural testing [14]. These order effects could even be amplified in animals with selective mutations in genes that are involved in physiological processes, such as coping strategies to changing environments. Circumvention of these interfering procedural aspects is required to reduce nonspecific environmental influences on the gene–behavioural phenotype relationship.
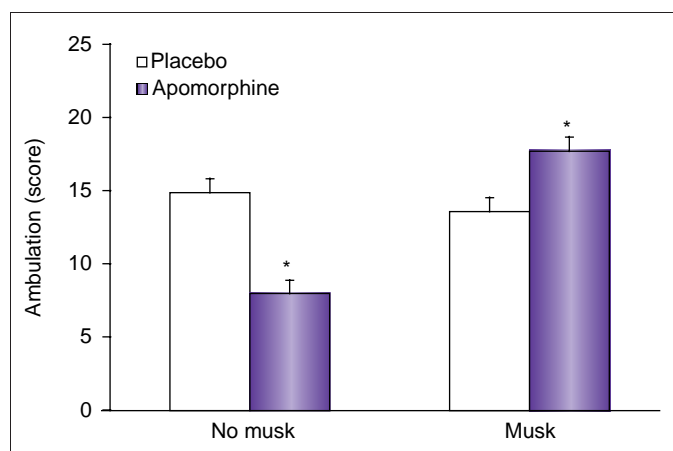
Dissection of behavioural phenotypes

Studies in the field of biological rhythms have revealed that behavioural observations during several consecutive days or weeks in the home cage of an animal allow reliable assessment of stable behavioural circadian rhythms that are highly sensitive to environmental signals, such as light and human interference [15,16]. Because behavioural observations during several days can also dissociate novelty-induced and baseline behaviours at different phases of the light–dark cycle, behavioural monitoring in the home cage will significantly contribute to the refinement of behavioural phenotypes. In addition, by carefully designing a home cage environment that addresses different behavioural characteristics of interest, complex behaviours can be further dissected into behavioural phenotypes with minimum human interference. For example, place preference and the expression of locomotor activity can be simultaneously measured by introducing shelter places in the home cage. Other behavioural parameters, such as

eating behaviour, can also simultaneously be dissected by recording meal frequency and duration. One should keep in mind, however, that some environmental factors can mask the expression of particular behaviours. For example, access to running wheels is often introduced as an easily detectable measure for circadian rhythms and locomotor activity levels in rodents, but wheel-running activity can significantly alter the amplitude of behavioural rhythms [17]. Thus, behavioural monitoring, including automated scoring, for multiple days in a home cage environment without confounding factors (e.g. masking effects), will further contribute to the refinement of behaviour and dissection of complex behaviours into different behavioural phenotypes within predefined working hypotheses.

Concluding remarks

In the post-genomic era, studies about the relationship between genotype and behavioural phenotype require novel methodology to measure behaviours. Because

Figure 2. Experimenter interference during behavioural assessment is a major confounding factor on the outcome of a behavioural test [12] and can even reverse the phenotype. Injection of a low dose of the dopamine agonist apomorphine (10 ng) into the nucleus accumbens of the brain decreased locomotor activity in rats (left). When the experimenter applied musk perfume to their wrists on the evening before the experiment, apomorphine induced hyperactivity (right) instead of hypoactivity. Asterisks indicate a significant different from placebo treatment. Reproduced, with permission, from Ref. [11].
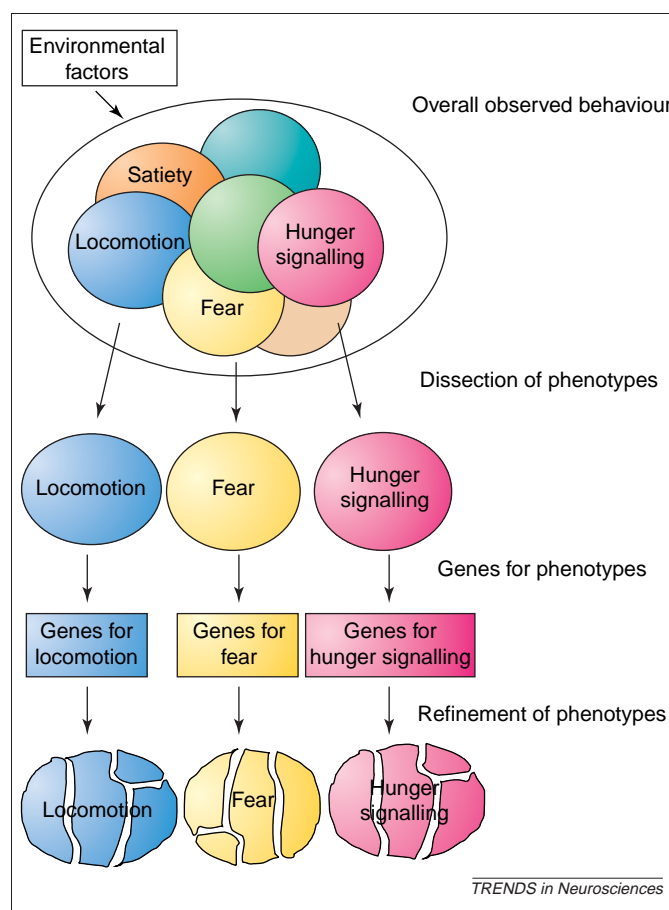
Figure 3. Determination of gene–behavioural phenotype relationships first requires dissection of the overall observed behaviour into behavioural phenotypes. The expression of the overall observed behaviour is likely to result from a complex gene–environment interaction [e.g. of molecules that regulate hunger signalling, fear, locomotion and other behavioural components (coloured ovals) with environmental factors such as food availability, ambient temperature and photoperiod variation]. Development of paradigms that allow dissection of complex behaviours into different behavioural phenotypes will optimize the search for genetic determinants for these phenotypes. Because genes influence brain region-specific aspects of a certain behavioural phenotype [20,21], local gene expression or deletion technology can further refine phenotypes when studied in environments closely related to the natural territory of the animal. In this way, a dissected phenotype such as hyperactivity can, for example, be further refined with respect to hyperactivity in nest-building behaviour or in food exploratory behaviour [21].

behavioural expression relies heavily on many inter-
actions between internal and external factors, working
hypotheses should direct the balance between environ-
mental conditions and recording duration, taking into
account the practical feasibility within the laboratory and
the behavioural resolution that minimizes false-positive
and false-negative findings. Inspired by the successful
identification of novel genes involved in circadian rhythms
[18,19], the use of automated behavioural measurements
for several consecutive days in the home cage of an animal
is put forward as a way to reduce significant contributions
of laboratory-specific variation and experimenter inter-
ference. This approach would also contribute to the
dissociation of interacting physiological processes, such
as novelty-induced and baseline behavioural activity, as
well as circadian variation in behavioural expression.
Multi-day recordings will, as a matter of course, reduce the
behavioural screening rate of a mouse colony. But, with the
improvements of behavioural resolution, it is also worth
considering a home cage environment that assesses all
behavioural components of interest at once, in contrast to a
behavioural screening program that entails multiple
short-lasting tests each addressing a single behavioural
component. Whether the focus of interest is cognition,
social activity, anxiety or eating, the development of
well-designed home-cage environments offers great oppor-
tunities for neuroscientists to optimize the dissection of
complex behaviours into behavioural phenotypes, and the
subsequent identification of the genes for these pheno-
types (Figure 3). Complex behaviour is influenced by
multiple genes, and one gene generally affects several
aspects of a complex behaviour. Interestingly, recent
studies on brain region-specific gene expression have
shown that a single gene can exert multiple functions
within a behavioural domain depending on the brain
region [20,21]. Thus, rodents with local vector-directed
gene expression or conditional knockout mouse lines
monitored in environments that address additional
aspects of their natural habitat (e.g. temperature and
photoperiod variations) will further optimize the search
for gene–behavioural phenotype relationships.

References

1 Hall, C.S. (1934) Emotional behavior in the rat I. Defecation and
   urination as measures of individual differences in emotionality.
   J. Comp. Psychol. 18, 385–403

2 Hall, C.S. (1936) Emotional behavior in the rat. III the relationship
   between emotionality and ambulatory activity. *J. Comp. Psychol.* 22,
   345–352

3 Pellow, S. *et al.* (1985) Validation of open:closed arm entries in an
   elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci.
   Methods* 14, 149–167

4 Crawley, J. and Goodwin, F.K. (1980) Preliminary report of a simple
   animal behavior model for the anxiolytic effects of benzodiazepines.
   Pharmacol. Biochem. Behav. 13, 167–170

5 Gerlai, R. (2002) Phenomics: fiction or the future? *Trends Neurosci.* 25,
   506–509

6 Wahlsten, D. *et al.* (2003) In search of a better mouse test. *Trends
   Neurosci.* 26, 132–136

7 Wisor, J.P. *et al.* (2001) Dopaminergic role in stimulant-induced
   wakefulness. *J. Neurosci.* 21, 1787–1794

8 Gainetdinov, R.R. *et al.* (1999) Role of serotonin in the paradoxical
   calming effect of psychostimulants on hyperactivity. *Science* 283,
   397–401

9 Dudley, C.A. *et al.* (2003) Altered patterns of sleep and behavioral
   adaptability in NPAS2-deficient mice. *Science* 301, 379–383

10 Drozdowicz, C.K. *et al.* (1990) Effect of in-house transport on murine
   plasma corticosterone concentration and blood lymphocyte popu-
   lations. *Am. J. Vet. Res.* 51, 1841–1846

11 Van Ree, J.M. and De Wied, D. (1988) Behavioral approach to the study
   of the rat brain. *Discuss. Neurosci.* 5, 11–60

12 Chesler, E.J. *et al.* (2002) Influences of laboratory environment on
   behavior. *Nat. Neurosci.* 5, 1101–1102

13 Crabbe, J.C. *et al.* (1999) Genetics of mouse behavior: interactions with
   laboratory environment. *Science* 284, 1670–1672

14 McIlwain, K.L. *et al.* (2001) The use of behavioral test batteries: effects
   of training history. *Physiol. Behav.* 73, 705–717

15 Daan, S. and Pittendrigh, C.S. (1976) A functional analysis of circadian
   pacemakers in nocturnal rodents. II. The variability of the phase
   response curve. *J. Comp. Physiol* 106, 253–266

16 Hastings, M.H. *et al.* (1997) Non-photic signalling in the suprachias-
   matic nucleus. *Biol. Cell.* 89, 495–503

17 Kas, M.J. and Edgar, D.M. (1999) A nonphotic stimulus inverts the
   diurnal-nocturnal phase preference in *Octodon degus*. *J. Neurosci.* 19,
   328–333

18 King, D.P. *et al.* (1997) Positional cloning of the mouse circadian clock
   gene. *Cell* 89, 641–653

19 van der Horst, G.T. *et al.* (1999) Mammalian Cry1 and Cry2 are
   essential for maintenance of circadian rhythms. *Nature* 398,
   627–630

20 Muller, M.B. *et al.* (2003) Limbic corticotropin-releasing hormone
   receptor 1 mediates anxiety-related behavior and hormonal adap-
   tation to stress. *Nat. Neurosci.* 6, 1100–1107

21 Szczypka, M.S. *et al.* (2001) Dopamine production in the caudate
   putamen restores feeding in dopamine-deficient mice. *Neuron* 30,
   819–828