Section 1 General

Chapter 1

Behavior genetics

Where do we come from and where are we going?

Wim E. Crusio and Robert T. Gerlai

Behavior genetics

Genetics is the science that studies the nature and action of genes, their transmission from parents to offspring, and their allele frequencies within populations. As Muller (1922) formulated it, "the question as to what the general principle of gene construction is . . . is the most fundamental question of genetics." This question has largely been answered and at present there is a huge amount of literature about the chemical structure of the genetic material, DNA, as well as vast databases containing the complete genomic sequences of an increasing list of organisms. Although (rather important) questions still remain, in principle at least the functioning of DNA is understood. This even led Caspari (1979) to conclude that "Genetics is in many respects a dead science." Obviously, many researchers living in the current exciting times where ever more detailed genetic information and increasingly sophisticated genetic tools become available probably disagree with this, but whether or not genetics has transcended from being a scientific endeavor in its own right to becoming an applied technology, the fact remains that it represents an elaborate and integrated system of well-established facts and theory that can be used by other fields of the life sciences, such as behavioral science. The traits studied by genetics are referred to as phenotypes (Crusio, 2002). Behavioral traits are one class of phenotypes and this choice of subject matter characterizes behavior genetics.

Although many consider behavior genetics to be a young field, one could regard Francis Galton (1822–1911) as the first behavior geneticist. However, one of the more explicit signs of the birth of behavior genetics as a separate scientific field was Hall's seminal chapter on psychogenetics in Stevens' *Handbook of Experimental Psychology* (1951). The starting point of behavior genetics is nevertheless more conveniently placed with the publication of Fuller and Thompson's book *Behavior Genetics* (1960), which indicated in the words of Lindzey et al. (1971) "a fully developed self-awareness of an important new specialty." In this chapter we will first discuss the focus of study of behavior genetics, behavioral phenotypes, followed by an exploration of the goals of the field and ending with a brief appraisal of how far we have come in realizing these goals.

Behavioral phenotypes

Fuller and Wimer (1973) divided phenotypes into two rather broad categories: somatophenes and psychophenes. This classification was extended by Fuller (1979b) and can briefly be outlined as follows. Firstly, we have somatophenes. These are characteristics such as body size and shape, pigmentation, etc. They are defined therefore by *structural* criteria. These somatophenes may be divided further into chemophenes, as for instance type of hemoglobin, and morphenes, such as body shape. Secondly, we have behavioral phenotypes, which sometimes also are referred to as psychophenes. These are measured directly or indirectly from behavior and are therefore defined by *process* rather than by structure. A further subdivision of psychophenes leads to the recognition of ostensible and inferred psychophenes. The former are based on the occurrence, frequency, and intensity of an objectively defined (behavioral) act. Inferred psychophenes are more general attributes or states of an organism such as anxiety levels and emotionality. The third and last category of phenotypes is formed by the syndromes. These are groups of psychophenes, usually occurring together with somatophenes. Some well-known examples include Down's syndrome, schizophrenia, and Fragile X syndrome.

Psychophenes and syndromes are the subjects of behavior genetics. Of these, inferred psychophenes and syndromes are usually the most difficult to define or interpret. The syndrome schizophrenia, for instance, is not always easy to delineate from related syndromes such as bipolar disorder (DSM-IV-TR, 2000; Kraemer et al., 2007), which of course can raise doubt about the justification of a patient's inclusion in or exclusion from an experimental group. Similarly, inferred psychophenes often give rise to interpretational difficulties, e.g., the concepts of "emotionality" (Fuller and Thompson, 1978) or "anxiety" (Stanford, 2007). Therefore, it is often safer to reduce the inferred psychophenes to the ostensible psychophenes on which they are based. Unfortunately, with the current emphasis on "translational" research, nowadays this is done only rarely and analyses abound of constructs such as "behavioral despair," "anxiety," etc. that are not always well defined (or even defined only

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operationally, hence the frequent discrepancies between results of different tests that are supposed to measure the same construct). On a more positive note, as stated by Fuller (1979a), "Perhaps this is . . . the very core of psychology. Here is where the action is. (But) . . . behavior geneticists should be aware of its inferential status and of possible arbitrary judgment in the definition of its members."

In selecting the appropriate psychophene for a behavior genetic study, several aspects should be kept in mind. The chosen phenotype should be one that can be measured reliably (and for economical reasons also with relative ease) and be of interest for ethological, psychological, and/or evolutionary theory (or as a model of a neuropsychiatric phenotype, the subject of Volume II of this Handbook). Furthermore, there are important advantages to choosing a measure of some elements of behavior that are relevant to the organism under natural conditions (Gerlai, 1999; Gerlai and Clayton, 1999) and, of course, at least some genetic variance (be it natural or induced) for the phenotype of choice should be present in the population studied (Fuller, 1979c; Henderson, 1979).

Aims and purposes of behavior genetics

The aims and purposes of behavior genetics have been formulated many times and by many different persons (mostly in the past: it appears that nowadays researchers are less inclined to spend time reflecting on the how and why of their chosen field of investigation). To start, Hall (1951) formulated four objectives of what he termed "psychogenetics": "(1) to discover whether a given behavior pattern is transmitted from generation to generation, (2) to determine the number and nature of the genetic factors involved in the trait, (3) to locate the gene or genes on the chromosomes, and (4) to determine the manner in which the genes act to produce the trait" (statements that still sound astonishingly modern, as do, in fact, large parts of the rest of Hall's chapter). Twenty years later, Thiessen (1972) posed eight questions for what had now become commonly called "behavior genetics": "(1) Is the observed behavior influenced by variations in genotype? (2) What proportions of the measured variability of a trait are the result of genetic and environmental factors? (3) Given a clear-cut genetic effect, how many genes are operating? (4) What is the frequency with which the gene appears within a population or a species group? (5) How is the gene modified by changes in the course of development or by environmental contingencies? (6) What structure and physiological processes intervene between the genetic constitution of an organism and the ultimate expression of behavior? (7) Does the trait have adaptive significance (that is, reproductive fitness), and is it subject to natural and artificial selection pressures? (8) What are the phylogenetic relationships of the behavior with related species?" Dewsbury (1978) condensed this to six very similar questions. Fuller and Thompson (1978) further reduced this to three basic questions: whether the psychophene is transmitted genetically, how the genes are distributed in space and time, and how the genes produce their behavioral effects. Finally, these questions were reduced to just two fundamental problems by van Abeelen (1979) who saw the goal of behavior genetics in the analysis of the phylogenetic as well as the phenogenetic causes of the psychophenes studied.

Following this, we can say that the ultimate aims of behavior genetics are twofold. The first aim concerns the investigation of the physiological substrates of psychophenes and the role of the environment therein (the phenogenetic aspect of the causation of behavior). At this point the profound influence of the environment on most of an organism's behavior must be stressed again. Not only are environmental effects one of the major sources of non-genetic variation, but genotype $\times$ environment interactions are also very important (Wahlsten et al., 2003). Therefore, the environmental contribution towards a psychophene is one of the major concerns of behavior genetics, too. The second aim of behavior genetics lies in analyzing the role of psychophenes in individual fitness, which of course includes the evolutionary history of the chosen behavior (the phylogenetic aspect of the causation of behavior).

Regardless of which one of these two aims was being addressed, genetics originally inquired about individual differences, that is, how differences between individuals come about either in a gene–physiological or in an evolutionary sense. Nowadays, the stress is often much more on the gene–physiological aspect, frequently completely ignoring individual variation: the question has become how genes lead to the expression of a certain psychophene, regardless of the question whether this psychophene is variable within the population or not.

However, the analysis of naturally-occurring genetic variation has important merits. First of all, the genetic differences represent physiologically relevant variation. The differences are obviously not so dramatic as to jeopardize viability and, more importantly, the reproductive fitness of the animal, and they represent conditions that have enabled the animal to survive successfully. Another advantage of analyzing naturally-occurring genetic variability is that by doing so one may be able to link the different variants to certain ecological conditions and/or explain the natural selection forces, i.e., the evolutionary past, which shaped the behavior in question.

Of course, genes that influence phenotypes with a high fitness component, i.e., those characteristics that are crucial from an evolutionary perspective, should show limited or no variability due to the strong selection pressures exerted on them (Broadhurst and Jinks, 1974). However, non-variable loci can nowadays also be studied using modern recombinant DNA technologies that allow the introduction of artificial novel mutations, approaches called reverse (or targeted) mutagenesis and forward (or random) mutagenesis.

How far have we come?

In the late 1960s and early 1970s many scientists still needed to be convinced that heredity could, in fact, influence behavioral differences between individuals. Proving that this was the

case was hampered by the fact that molecular–genetic techniques enabling the localization of genes were still rudimentary or non-existent. However, by the late 1980s and the early 1990s, almost all serious scientists had come to accept the importance of genetics for understanding interindividual variation in behavior (Plomin et al., 2003). As may be expected, this realization occurred earlier in animal genetics than in human genetics. However, once this realization had taken root, it became possible to expand behavior–genetic research beyond the simple calculation of heritability (h^2, the proportion of phenotypical variance in a population that can be attributed to heredity). This was an important advance, since "heritability analysis" in itself is not very interesting or useful, apart from the questions whether h^2 differs significantly from 0 (showing that significant genetic effects are present) or from 1 (demonstrating that significant environmental influences are present). In parallel with the enormous advances in molecular genetics, it has now become increasingly feasible to investigate the *mechanisms* underlying interindividual differences and identify and analyze the underlying genes, the subject matter of the current volume. In addition, going beyond classical methods such as genetic selection and random mutagenesis, it is now possible to modify genes in animals in a targeted manner by inserting foreign genetic material into the genome in such a way that it gets expressed or by "knocking out" specific genes. The latter methods have become invaluable tools in investigating the gene–physiological bases of behavior and, in addition, have made it possible to create pertinent models for single-gene disorders, the subject matter of Volume II of this Handbook.

Where are we going?

Predicting the future course of a field of scientific endeavor is always a risky undertaking at best and futile at worst. It is difficult to impossible to know for certain what new methods and techniques will become available even in the near future and, even when they are already in existence, it is often nearly impossible to correctly predict their impact. A case in point is the enormous growth of molecular–genetic methods. In the late 1980s and early 1990s, many predicted that it would now become relatively easy to localize genes for complex characters such as behavior. The Human Genome Project was touted to lead to cures for many devastating disorders within years of the completion of the sequencing of the human genome. Of course, by now we know that this has not been the case. As so often, reality has shown that things are more complicated and more difficult than we thought (or hoped) in our initial enthusiasm, and preciously few genes have been identified for any behavior, be it in mice, humans, or other organisms. Indeed, understanding how genes influence brain function and behavior is a much more complicated endeavor than originally forecast. The danger is that by creating such false expectations, we risk losing our scientific credibility with society at large and, in consequence, with policy makers. History up till now has taught us to be careful in our future expectations.

The above notwithstanding, we feel that some predictions can be made with a certain level of confidence. The arrival of new genetic tools, such as the expanded set of BXD Recombinant Inbred Strains (Peirce et al., 2004) or the Collaborative Cross (Churchill et al., 2004), may finally allow us to identify some of the genes responsible for the myriad of quantitative trait loci (QTLs) that have been localized in the past two decades. Similarly, after many years of churning out huge streams of gene-expression data, DNA microarray technology along with advances in bioinformatics is now slowly morphing into a new field called systems genetics (Schughart and SYSGENET consortium, 2010) with promising new tools to enhance our understanding of how the manifold interactions between genes cause interindividual variation. The increasing sophistication with which cell-type-restricted and temporally controlled gene targeting may be performed appears also promising, as do novel technologies including the utilization of miRNAs or RNA interference. These and many other methods will undoubtedly allow behavior geneticists to arm themselves with increasingly precise and controlled genetic tools. Obviously, the end of history has not yet been reached in behavior genetics.

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Section 1 General

Chapter 2

Natural neurobiology and behavior of the mouse

Relevance for behavioral studies in the laboratory

Hans-Peter Lipp and David P. Wolfer

Summary

The house mouse (*Mus musculus*) has its origins presumably in Asia. Among many other small rodents, it represents an extremely flexible and adaptive species. Commensalism with human civilization and agriculture resulted in a worldwide distribution. While biology and behavior in the laboratory are well-documented, ecological–behavioral studies in natural or naturalistic environments are comparatively rare.

The relevance of the natural organization of behavior for laboratory tests appears to depend on an intracerebral hierarchy of sensory abilities and related behavioral processing crucial for survival: defensive fear-related behaviors, exploration and foraging strategies, olfactory communication and reproductive behavior, behavioral flexibility, and, lowest in the hierarchy, cognitive processing and complex memory. The relative lack of higher-order associative cortex in the house mouse also implies that the mouse hippocampus and prefronto-limbic cortex remain as the main associative structures, yet predominantly orchestrating ethologically relevant processes. Thus, experimental and genetic manipulations of the mouse brain for behavioral analysis need to consider its evolutionary adaptations and constraints.

These ideas shall be illustrated with some examples of outdoor studies in mice.

Introduction

The house mouse (*Mus musculus* ssp.) represents, as humans, rats, and sparrows, a recent evolutionary success story (Bonhomme et al., 1984; Bronson, 1984). From its origin in the Indian subcontinent some 500 000 years ago, this species ramified into the ancient Middle East from where it spread all over the world, following humans to almost every place except the arctic regions (Boursot et al., 1993). The reasons for its ability to follow humans (commensalism) and for its remarkable capacity to adapt to a large variety of habitats not shared with humans remain largely unknown (Berry and Bronson, 1992; Frynta et al., 2005). Its domestication, initially by fanciers, and much later on by scientific institutions, makes it the most frequently used laboratory animal at present.

While the mouse remains probably the best-investigated species with respect to genetics, cell biology, and physiology, knowledge about its brain and behavior is comparatively rudimentary, despite the many reports of the behavior of genetically modified animals. Much of that knowledge is inferred from rats: studies elucidating brain-behavior mechanisms in mice themselves are not abundant. Part of this problem is the paucity of ethological studies in naturalistic and semi-naturalistic environments, a prerequisite for meaningful interpretation of phenotypic changes in transgenic mouse models (Gerlai and Clayton, 1999). Thus far, behavioral studies of wild mice in naturalistic environments are rare, and appear to be of little interest as evident by the neglect of the beautiful monography by Crowcroft (1966). Most of them have focused on reproductive biology (Bronson, 1979; Drickamer et al., 1999, 2000), others on habitat structure (Pennycuik et al., 1987; Plesner-Jensen et al., 2003), on behavioral mechanisms underlying fluctuations of population densities (Van Oortmerssen, 1971), and on effects of predator pressure on populations (Arthur et al., 2005). Even less frequent are studies on learning abilities and behavioral traits of normal and experimentally manipulated laboratory mice and strains in naturalistic environments (Blanchard and Blanchard, 2003; Dudek et al., 1983; Glickman and Morrison, 1969). Clearly, the necessity of such approaches has been recognized by many behavioral scientists, but observing mice in naturalistic environments has been technically difficult and tedious.

Natural constraints for behavioral phenotyping in genetically modified mice

A casual survey of the many papers describing behavioral phenotypes of genetically modified mice reveals a conundrum: the majority of them describe altered hippocampus-dependent behaviors irrespective of whether the mutation was specific for hippocampal neurons or ubiquitously expressed in the brain. Quite often, hippocampus-dependency is also taken as a synonym for cognitive changes, particularly so if the modified gene is considered as being important for cellular processes underlying memory formation, and the mouse line is intended to serve as a model of human psychopathology. On the other

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hand, closer inspection of such reports reveals that the observed changes not only include popular hippocampus-dependent tasks, such as the water maze, the radial maze, and contextual fear conditioning, but also "exploratory" inspectional behavior observed in object recognition tasks and spontaneous alternation in T-mazes. Thus, while not evident from reading a single paper, a comprehensive view conveys the impression that most genetic manipulations result in behavioral manifestations linked in one way or the other to hippocampal function. Another puzzling finding is that when mice are tested for social behavior, in particular intermale aggression, there appears to be a disproportionably high number of targeted mutations enhancing or reducing aggression with no obvious relation to brain structures known to regulate murine aggression (Maxson and Canastar, 2003; Miczek et al., 2001). Similarly, many transgenic mouse lines show up- or down-regulation of open field activity.

In order to understand this, it is necessary to recall the natural constraints of phenotypic expression of mutations, as well as of lesions and pharmacological manipulations. These constraints include: (1) the species-specific (ethological) behaviors that a mouse employs to cope with natural or test situations in the laboratory; (2) the species-specific neural output pathways for translating brain processes into behavior; (3) the role of the hippocampus and limbic system in orchestrating species-specific behavior; and (4) the evolutionary pressure modeling and ordering different neural circuits according to their relevance for survival and biological fitness of *Mus musculus*. The following paragraphs shall try to sketch these points.

Ethology

It is undisputed that the behavioral repertoire of the house mouse ought to be considered when interpreting behavioral changes following experimental manipulation of the brain. Despite the efforts of ethologically oriented behavioral scientists such as Bolles (1970), Blanchard and Blanchard (1988), and, for genetically modified mice, Gerlai and Clayton (1999), their caveats are too often not considered. For example, it is well known that the defensive response of mice to natural and learned threatening stimuli includes freezing, flight, and risk assessment, and that mice can shift, depending on the situation and stimulus, rather flexibly from one of these behaviors to another. Nonetheless, most studies employing contextual fear conditioning (during which a mouse is placed in an environment where it previously received signaled punishment) use only the time spent freezing as an equivalent of a memory trace. While the presence of freezing indicates qualitatively a memory trace, quantitative variation of this freezing response may equally indicate either a decay in memory or else a shift in response pattern as the mouse tries locate the threat or to get out of that environment after some time. Thus, changes in very different brain mechanisms may result in similar reduction of freezing.

Neural output

A subtler point is how brain processes translate into behavioral events. To recall a basic feature: regardless of the species-specific adaptation of the brain for sensory and cognitive abilities, the endpoint of all cerebral processing in vertebrates is contractions of striate and smooth muscles. The former manifest as species-specific changes of body parts or movements across space, the latter as changes in the autonomous nervous system, being mostly invisible, less frequently also apparent to an observer as piloerection, changes in skin color, or secretion of pheromones, to name a few. These two output pathways are found in all vertebrate species, the neural programs for activation and inhibition being located in the reticular formation of the rostral brainstem. The midbrain controls species-specific behavioral events in form of a stop-and-go principle, summating facilitatory and inhibitory signals according to locally stored memory, exteroceptive (visual, somatosensory, auditory, and olfactory) and interoceptive (hormonal and gustatory/chemical) inputs. Hormonal information from the hypothalamic receptors and olfactory input reaches the midbrain through a chain of reciprocally interconnected structures and axons, enabling a coarse evaluation of rewarding properties (go signals) along the laterally running medial forebrain bundle, while aversive/alarming signals are conveyed via more medially running fibers to the central gray and brainstem nuclei of the autonomous nervous system. Taken together, the ultimate behavioral control is governed by stop-and-go signals originating in the midbrain, but there are important species differences in the modulation of these processes, depending on the degree of forebrain development (encephalization). This is most evident by considering the neuroanatomical differences between mice and men.

Hippocampus of mice and men

The main difference between the mouse (or rodent brain in general) and the primate brain is that the limbic output from prefrontal cortex, hippocampus, and amygdala acts more directly on subcortical motor systems activating the midbrain stop-and-go system than in primates. This is due to two connective properties. For one, in rodents, a considerable portion of the limbic output from prefrontal cortex, hippocampus, and amygdala terminates in dorsal and ventral basal ganglia whose output is directed chiefly to the rostral brainstem, inhibiting or activating ongoing species-specific motor acts. In primates, the output of the basal ganglia converges preferentially on the motor thalamus and thus on the primary motor cortex, whereas output from limbic basal ganglia reaches the midbrain and the intralaminar thalamus, which in turn may control neocortical processing by ascending systems (Lipp and Wolfer, 1998). Thus, motor activity in humans reflects eventually the neural activity of the entire neocortex, whereas motor activity in rodents reflects primarily limbic processing acting on midbrain structures. Consequently, large portions of the rodent neocortex can be

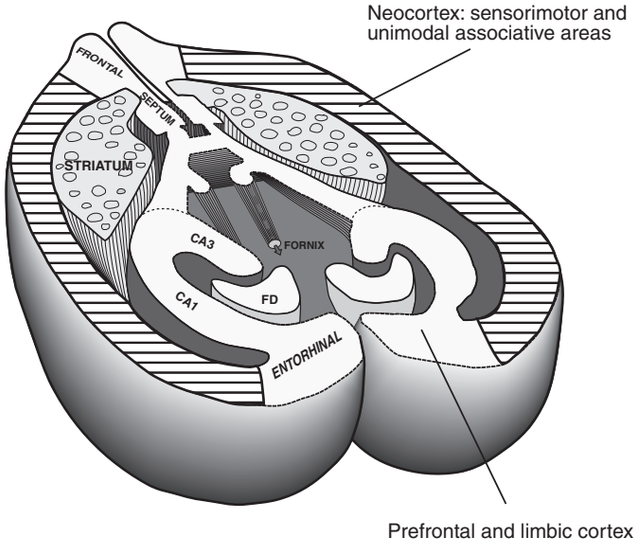

Figure 2.1 Schematic view of a horizontal section through the mouse brain. Hatched areas denote neocortex devoted to sensory, motor, and modality-specific analysis. Note that the hippocampal formation and the (small) prefrontal areas remain as the main higher-order associative cortex.

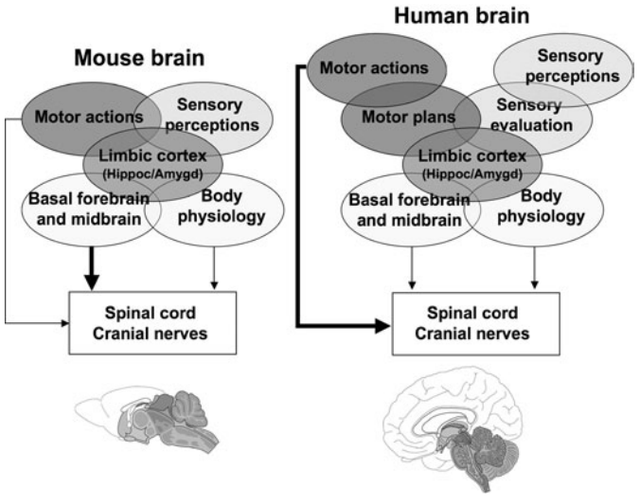

Figure 2.2 Organization of behavioral output pathways in mouse and human brain, and the central role of the limbic cortex which, in the mouse, directs its outputs preferentially towards the midbrain. Thus, most processing in the forebrain results in immediate motor reactions. In humans, large portions of the behavioral output system are shifted towards the motor cortex, the limbic system acting on the neocortex, both through mesencephalic and thalamic feedback loops and reciprocal connections with higher-order associative cortex. This causes iterative processing, resulting in constant adaptation and thus less abrupt changes of ongoing motor activities. In terms of inputs, the mouse hippocampus receives motor and sensory information, without much preprocessing through higher-order associative areas as observed in the human brain.

removed without disabling gross motor activity (Figure 2.1) (Huston and Borbely, 1973).

The second connective difference is the relative lack of polymodal (higher-order) associative cortex in rodents, most clearly seen in mice in which most of the neocortex comprises primary sensory areas and unimodal association cortex (Figure 2.2). In essence, the hippocampus is their main associative cortex, linking both pre-processed sensory and motor information. It is thus likely to be (variably) involved in any kind of complex learning. In humans, the hippocampal formation connects primarily with polymodal motor (executive) or polymodal sensory neocortex (Figure 2.2). Hence, lesions or malfunctions of the human hippocampal formation and proximally connected limbic areas manifest themselves as deficits in memory or cognition but have little impact on ongoing motor behavior except for verbal communication. On the other hand, malfunctions of the mouse hippocampus and associated limbic structures will result in *both* impaired orchestration of species-specific motor responses in the midbrain, evident as hyper-reactivity plus movement stereotypies, and impaired spatial abilities. This is because the only substrate for finely matching directed movement with sensory information has been disabled. One may note that this view would predict the presence of place cells in the rodent hippocampus but the relative paucity of such cells in the hippocampus of monkeys or humans.

Taken together, a certain amount of "hippocampal" behavioral impairments in mice is likely to occur in many transgenic mouse models showing a behavioral phenotype. They may be equally observed after specific inactivation of hippocampal substructures, after ubiquitous impairment of neuronal function in the forebrain, or even in mutations sparing the hippocampus. This happens because most efferent fibers of the mouse forebrain are targeting rather directly the same stop-and-go system in the midbrain, and because the mouse hippocampus interacts primarily with modality-specific parts of the neocortex. Thus, these behavioral signs cannot be taken as an indicator of cognitive malfunction or memory impairments in the human sense, but are a relatively fine indicator of neuronal malfunction within but also outside the hippocampus. In practice, employing "hippocampal" tests for behavioral phenotyping of genetically modified mice is useful for screening but of limited value for testing psychological concepts.

Ecological constraints

The frequently observed up- and down-regulation of putative "non-hippocampal" behaviors such as aggression or open field activity indicates another interpretation problem. If observed in a given mutant line, there is often a penchant to attribute this to malfunction of a particular neural subsystem regulating that behavior. However, such interpretations neglect the fact that most subsystems regulating species-specific behaviors (via the midbrain stop-and-go system) interact homeostatically, particularly those competing antagonistically for a motor output requiring approach, avoidance or immobility. This is most evident in hypothalamic brain stimulation studies capable of activating simultaneously rewarding and aversive neural subsystems (Lipp, 1978, 1979). In terms of genetic manipulations,

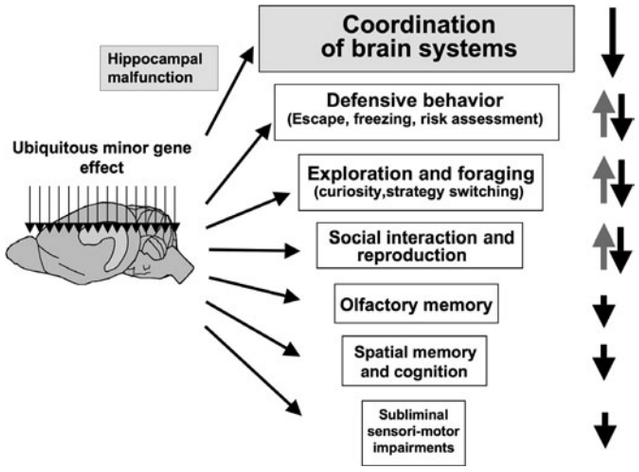

Figure 2.3 Hierarchy of expected behavioral phenotypes of murine mutations that have ubiquitous action in the central nervous system, or entail extensive pleiotropy, arrows to the right indicating up- or down-regulation. "Hippocampus-dependent" behaviors are expected to occur most often, alone or in combination, because of the size of hippocampus, its role as largest associative brain region, and because of many other brain regions mimicking hippocampal function by jointly acting on the midbrain stop-and-go system. The other potential effects of ubiquitous mutations on specific behavioral phenotypes have a likelihood decreasing in parallel with their ecological and functional significance. Thus, the next likely candidates for phenotypical up- or down-regulation are changes in the balance between defensive and exploratory behavioral tendencies, possibly also behavioral flexibility versus rigidity, followed by up- or down-regulation of aggressive behavior and social interactions. Because of the importance of smells for the daily life and reproduction of mice, unspecific mutations affecting concomitantly the olfactory systems have also an increased likelihood of phenotypical manifestation in olfactory-dependent behaviors, albeit less likely as gain-of-function. Specific effects on spatial memory and learning may occur but confounds with neural systems co-mediating the midbrain stop-and-go system are to be expected. Finally, there might be many subtle sensori-motor deficits yet difficult to observe at the behavioral level.

this implies that up- or down-regulation of particular behaviors may often reflect an altered homeostatic balance between neural subsystems rather than alteration of a given neural subsystem. Obviously, behavioral observation alone cannot discriminate between the two possibilities.

Yet, it is reasonable to assume that natural selection is carefully tuning the balance between such systems according to species, ecological niche and even individual propensities within a population. This is indicated by the very rapid effects of natural selection in both mutant mice and mice carrying natural genetic variability (see below), and also by the observations that many targeted mutations entail, somewhat unpredictably, up- or down-regulation of behavioral traits seemingly unrelated to the targeted mutation. In mice, it would seem that there is a hierarchy of such processes according to the importance for survival and biological fitness.

This shall be exemplified by assuming a targeted mutation with ubiquitous but minor effects on neuronal functioning across the entire forebrain (Figure 2.3). Because of its relatively large size, a certain degree of hippocampal malfunction is likely to occur. This will be preferentially reflected in mal-coordination of spatial behavior but also in shifted balances between antagonistic systems governing species-specific behavior. In addition, the balance between ecologically important behaviors is likely to be tuned additionally by non-hippocampal systems. For mice as a small and highly predated species, the most important behavioral system is the one regulating the reactivity to external stimuli (many of them potentially threatening) and the selection of antagonistic defensive behaviors; that is, immobility versus flight, because this determines life or death. Depending on the local situation, either behavior can be appropriate. Thus, mutations with general effects may increase or decrease the propensity for running versus freezing (and might so be mistaken as up- or down-regulation of memory in contextual fear conditioning).

A second class of antagonistic behavior is the propensity of exploring and foraging necessary to locate food, which, however, bears an increased risk of predation, and must thus be subject to a carefully tuned check-and-balance system resulting either in more curious or more fearful animals. Again, a shifted balance may be mistaken as increased genuine curiosity or fear, respectively.

While less important for daily survival, social interaction and reproduction are of paramount importance for a short-living social species. Particularly in male mice, many minor genetic disturbances do have the potential to alter the balance between attack and flight, thereby affecting social status. This might explain why so many mouse mutants appear hypo- or hyperaggressive.

On the other hand, the functional relevance of memory processing may be of lesser importance for a species with a lifespan that, under natural conditions, rarely exceeds 6 months. Given that male mice distribute daily up to 40 mg of major urinary proteins (Beynon and Hurst, 2004; Hurst and Beynon, 2004), one would expect that olfactory memory mechanisms are by far the most important ones for these species. Likewise, olfactory processing is critical for the survival of pups.

Surprisingly from a psychologist's point of view, yet unsurprising for ethologists, spatial memory and cognition do not appear to be of tremendous importance for a species preferring to move in a well-known, spatially confined, and mostly dark environment along olfactory paths

Finally, one might expect that a mutation acting ubiquitously in the central nervous system of a mouse is likely to impair a variety of sensory and motor processes. However, if the mutation does not have a strong effect, a phenotype may be difficult to detect. For example, an overall decrease of 20% in synaptic transmission in all neurons may entail phenotypic changes at the behavioral level. However, measuring concomitant minor impairment in sensory or motor processing would need extensive behavioral and neurophysiological studies to document it.

The next sections describe an approach of how to study the effects of genetic and classic lesions on brain and behavior of mice living in semi-naturalistic environments, and they will illustrate some of the theoretical points made above.

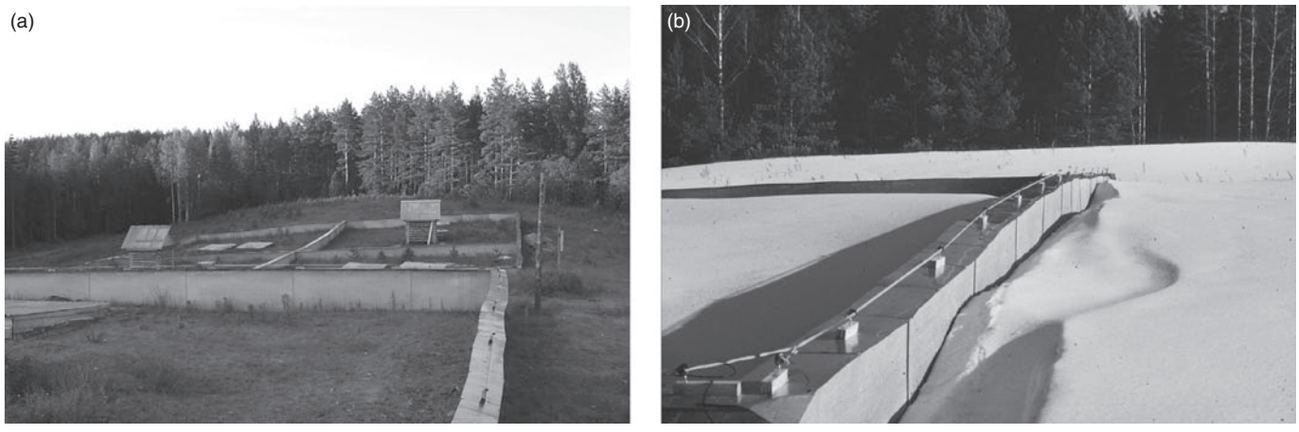

Figure 2.4 Outdoor pens for studying learning and natural selection in laboratory mice in summer (a) and winter (b).

Studying behavior and survival of mice in outdoor settings

Principles

One procedure suitable to test the effects of genetic manipulations on general biological fitness and ecologically relevant behaviors is to release mice into outdoor settings for a limited period during summer and early fall. Provided that spacious shelters are available, fluctuating meteorological conditions in this time are well tolerated by mice, except by some inbred strains (see below in this section). On the other hand, the mice are at risk for aerial predation as soon as they leave the protected shelters and pathways leading to outdoor sites, and they have to face a tremendous change in environment. This offers a convenient opportunity for testing whether behavioral changes observed in the laboratory predict behavior in naturalistic environments, and also for testing whether the mice suffer from unrecognized maladaptive effects of mutated genes.

If the interest is on natural selection rather than short-term adaptation, mice genotyped for modified and wildtype alleles are released in proportions matching Mendelian inheritance (e.g., the founder population includes 25% homozygous mutants, 50% heterozygous, and 25% wildtype mice, resulting in a balanced distribution of 50% wildtype and 50% mutant alleles). The mice are then left with food *ad libitum* and recaptured every year for genotyping, being re-released afterwards. This approach permits observing natural selection effects on brain and behavior, or the elimination or accumulation of targeted mutations in the offspring of the released animals.

The simplest method for assessing the impact of a treatment or of a genetic mutation is re-trapping ear-tagged mice after a defined period, be this after a few weeks, or just every year. A more sophisticated method is animal monitoring by using implantable passive radio-frequency identification (RFID) transponders (Dell'Omo et al., 1998, 2000).

In cooperation with Russian behavioral geneticists at Moscow State University, we had the opportunity to build a field station for studying the effects of natural selection on artificially mutated genes, brain traits, and associated behavior, and also the effects of genetic manipulations and experimental lesions on behavioral abilities in naturalistic environments. For this purpose, the field station contained several large outdoor pens (Figure 2.4) and a field laboratory permitting local neurohistology of mice. This chapter will review exemplary studies that permitted us to identify main determinants and species-specific constraints of the behavior of the house mouse in naturalistic conditions.

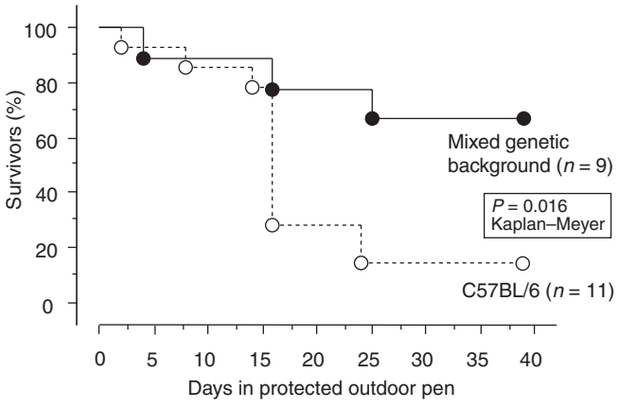

Figure 2.5 Short-term survival of female C57BL/6 and mice of mixed genetic background (random-bred from diallel cross C57BL/6J, C3H/J, NZB/J, and DBA/2J). The curves include both mice with hippocampal lesions and control mice. Presence of mice was monitored by means of subcutaneously implanted microchips.

Short-term survival of normal inbred strains

Pilot studies and short-time experiments clearly indicate that hybrid mice with mixed genetic background always adapted easily to summer conditions, while this was not always true for inbred strains. The effects of mixed versus inbred genetic background is evident in Figure 2.5 showing more severe losses of female C57BL/6 mice as compared to random-bred mice,

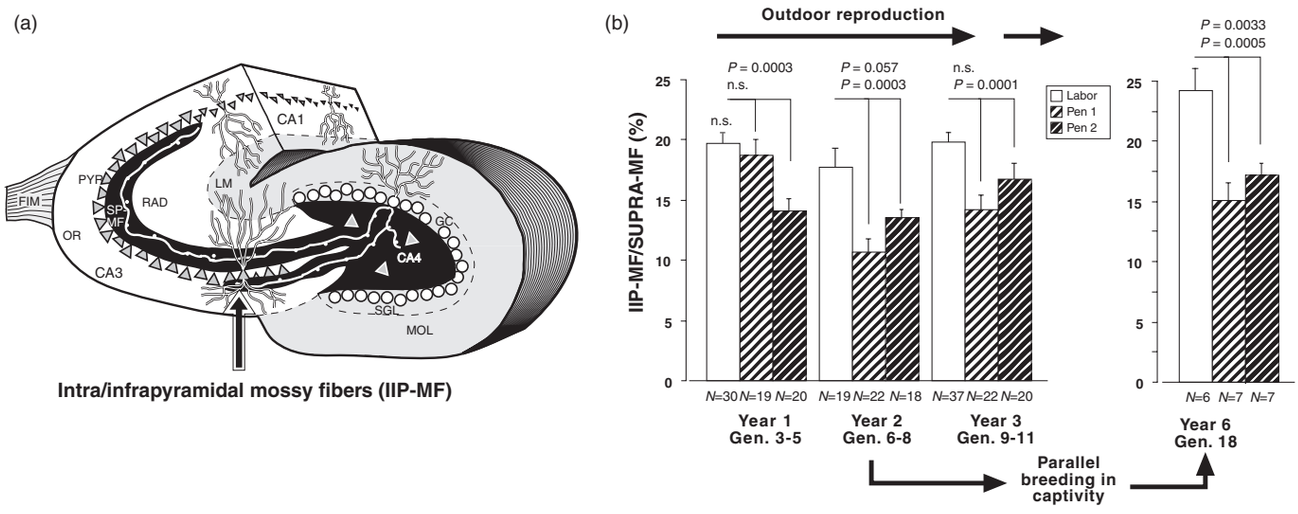

Figure 2.6 Intra/infrapyramidal mossy fiber (IIP-MF) projections and natural selection over 3 years in outdoor pens. (a) Diagram of mouse hippocampus with mossy-fiber projections. (b) Reduction in the extent of the IIP-MF projections as observed during 3 years of living in outdoor pens. The reduction of the IIP-MF in the feralized mice remained after having transferred the mice to mouse facilities and through embryo transfer, which indicates natural selection.

even when the enclosure was additionally protected with a net against aerial predators. These mice had food *ad libitum*, suffered no bad weather, had empty space in two shelters, and no or little social stress. Thus, the perhaps most simple conclusion from these adaptation studies is that inbred strains are likely to carry maladaptive traits not evident in the laboratory. Consequently, our laboratory avoids behavioral testing of mutant mice on an inbred background and prefers testing hybrids according to the Banbury recommendations (Wolfer et al., 2002).

When male and female mice are released together, the number of male mice in unprotected outdoor pens decreased generally faster than the number of females, regardless of genotype. This can be expected from the social structure of mice characterized by dominant males chasing subordinates relentlessly also in naturalistic settings (Crowcroft, 1966; Ely et al., 1976). Thus, dominant males tend to occupy the protected shelters, presumably forcing subordinates to enter unprotected risk areas more frequently. Therefore, the cerebral regulation of intermale aggression is one of the decisive mechanisms in both short-term adaptation studies of individual mice and multigeneration studies on natural selection.

Long-term selection of hippocampal mossy fiber traits and associated behavior

Background

Another line of research in natural selection originated from the discovery that hereditary variation of a hippocampal structural trait in rats and mice appeared to be correlated with learning abilities in laboratory tasks (Schwegler and Lipp, 1983). Hippocampal mossy fibers are the axons of dentate granule cells terminating in defined layers above and below the pyramidal target neurons in hippocampal subregion CA3 (suprapyramidal (SP-MF) and intra/infrapyramidal mossy fibers (IIP-MF) respectively; Figure 2.6a). Somewhat surprisingly, the extent of the IIP-MF projection along the basal dendrites was often correlated with performance in a variety of hippocampus-dependent tasks; for example, negatively with two-way avoidance learning, and positively with radial maze learning and the efficiency of platform reversal learning in the water maze. This had been verified in a long series of studies using strains selectively bred for extremes in behavior, inbred, and random-bred strains of mice, and ontogenetic manipulations of the IIP-MF projection (for reviews see Crusio and Schwegler, 2005; Lipp et al., 2006). In many cases, reduced IIP-MF projections appeared to mimic a mild hippocampal lesion (also known to improve two-way avoidance learning), while extended projections appeared to be associated with a factor reflecting an intact basic hippocampal function necessary for complex (mostly spatial) learning. However, the extent of the IIP-MF appeared also to be correlated with behaviors not considered as hippocampus-dependent, such as strength of paw preference being more pronounced in mice with large IIP-MF projections (Lipp et al., 1996), and reduced attack latencies as observed in intermale aggression in mouse strains with small IIP-MF projections (Guillot et al., 1994; Sluyter et al., 1994). These latter observations provided a hint that the mouse hippocampus might be mediating behavioral mechanisms not necessarily predicted by the human hippocampal lesion syndrome. Our explanatory hypothesis was that structural mossy fiber variations might pre-set individual behavioral reactivity to distracting stimuli of exteroceptive or interoceptive origin. Thus, small IIP-MF would be associated with short attack latencies and superior two-way avoidance learning (requiring immediate motor reaction as operant response), while such high reactivity would be detrimental for most complex learning tasks requiring attention and suppression of inappropriate responses.