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Behavioural screening in mutagenised mice—in search for novel animal models of psychiatric disorders

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Abstract

Complementary to the 'gene-driven' analysis of gene function, 'phenotype-driven' approaches can be performed and may be equally important. Despite the current availability of a long list of mouse mutants, there remains an appreciable need for behavioural phenotypes in mouse models permitting to learn more about the aetiology of psychiatric disorders. This lack can be compensated by phenotype-driven ethyl-nitrosourea (ENU)-mutagenesis programs which aim at identifying novel phenotypes without any a priori assumptions, thus, representing a unique possibility to create novel animal models which approximate the underlying genetic aetiology. The power of mouse mutagenesis critically depends on the phenotyping procedures performed. In the case of ENU-mutants, behavioural phenotyping is especially challenging, as behavioural profiles have to be identified in single individuals. For high-throughput screening, approaches have been made to establish standardised screening protocols including a combination of well-validated, easy to perform behavioural tests. Different strategies are being introduced, which are used in ENU-mutagenesis screens to identify behavioural mutants representing possible endophenotypes of psychiatric diseases.
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1. Introduction

1.1. Identifying candidate genes for psychiatric disorders

Rational strategies for the advancement of treatment strategies are dependent on improving our knowledge of those genes that contribute to psychiatric disorders and the neural pathways altered by psychotropic agents. We, therefore, have hopes that application of genomic technologies to pedigree and population samples of patients with psychiatric disorders will allow the identification of genes contributing to the aetiology and pathogenesis of these devastating diseases in order to provide a rational basis for new drug development. With single gene disorders, i.e., mendelian disorders such as cystic fibrosis or Huntington's disease, there is a simple, direct relationship between variation in a single gene and the resulting phenotype. In contrast, the relationship between phenotype and genotype is not that straightforward for complex genetic traits. In this setting, multiple different susceptibility genes and environmental factors interact in varying combinations within individuals who appear to have clinically indistinguishable phenotypes, i.e., a psychiatric diagnosis based on the current categorical classification systems such as ICD-10 or DSM-IV. Attempts to first map and then identify genes predisposing to psychiatric disorders, therefore, have been frustrating with respect to the complexity of the genetic mechanisms underlying behavioural phenotypes (Kendler, 2002). So far, only very few genetic factors could consistently be implicated in the pathogenesis of psychiatric disorders such as depression using standard linkage analysis (see Hyman, 1999; Owen et al., 2000 for review). These inconsistencies are likely to be explained by the genetic complexity of these disorders. Relatively common polymorphisms in several genes may coincidentally contribute to an increased susceptibility for affective disorders, each polymorphism only adding a comparatively small increase in relative risk. In humans, genetic linkage analysis assessed in population and family-based studies revealed genetic polymorphisms mainly within classical neurotransmitter

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systems (e.g., Heils et al., 1996). In order to understand the complex interaction of multiple gene loci, new methods based on single nucleotide polymorphism analysis have been successfully established. These polymorphisms consist of a single base exchange and occur every 500–1000 base pairs. Single nucleotide polymorphisms can either be functionally relevant themselves or serve as markers for other nearby mutations with which they are in linkage disequilibrium. The knowledge of the human genome and the development of high-throughput techniques have enabled us to this specific approach. Due to the still existing limitations in throughput techniques and the costs involved, a hypothesis-free complete genome screen, i.e., genotyping of up to 100.000 markers in thousands of individuals, amounting to a total of millions of single nucleotide polymorphisms, is not yet realisable (Carlson et al., 2001). For this reason, genetic investigations still focus on candidate gene association approaches. Taking into consideration certain caveats, such as careful phenotyping of the investigated population and intelligent selection of candidate genes/candidate pathways and single nucleotide polymorphisms within these genes, this method is very powerful in the genetic dissection of complex traits underlying psychiatric disorders (Tabor et al., 2002).

So far, studies on genes other than those involved in monoaminergic drug action or metabolism, including the influence of the long and short alleles of the serotonin transporter on the response to selective serotonin reuptake inhibitors, have shown controversial results (e.g., Kirchheiner et al., 2001). Thus, approaches are required that are not related to molecules directly targeted by the known antidepressant drugs, as these are likely to be linked only indirectly to the mechanisms responsible for clinical improvement (Holsboer, 1999). To this end, there is a great demand for suitable animal models, in which novel genes playing a role in behaviour may be identified and analysed systematically in a subsequent candidate gene association study approach in patient populations. Large-scale mutagenesis represents a possibility to create animal models, which approximate the underlying genetic aetiology. In this context, the ethyl-nitrosourea-mutagenesis approach is considered to be a rich resource for identifying novel candidate genes and single nucleotide polymorphisms of behavioural abnormalities.

1.2. Ethyl-nitrosourea mutagenesis

Although a large number of genetically engineered mouse mutants will be available in the near future, genetic analysis requires the availability of multiple alleles of the same gene or of different genes involved in the pathogenesis of the same disease, including hypomorphs, alleles of different strength, and gain of function alleles. Such alleles can be obtained after treatment with chemical mutagens, such as ethyl-nitrosourea (ENU), which is suggested to be the most powerful mutagen in mice. In contrast to, for example, radiation, the alkylating agent ENU induces primarily point mutations with mutation rates of about one new mutation per gene in every 700 gametes (Balling, 2001). The characteristic of this substance used for mutagenesis programs is its efficiency in mutagenising male premeiotic spermatogonial stem cells in mice, leading to a large number of F1 animals carrying different mutations. Notably, inbred mouse strains show a remarkable variability in their response to treatment with ENU (Justice et al., 2000). Moreover, since ENU is not only a mutagen but also toxic in nature, a careful treatment regime has to be established for successful large-scale mutagenesis programs.

Given the above-mentioned mutation rate, it can be assumed that quite a large number of mutations are induced in every ENU-mutant. It has been calculated that 25 to 40 distinct mutagenised genes may be present in one ENU-individual (Balling, 2001) and, consequently, it has been argued that the observed phenotype of ENU-mutants is likely to represent multigenic traits, which would of course be extremely difficult to identify. To exclude phenotypes, which in fact are inherited altogether, ENU-mutants are bred for several generations since it is extremely unlikely that genes underlying a multigenic phenotype will segregate together. Thus, only ENU-mutants that show a stable phenotypic penetrance in their offspring over several generations will be further investigated.

The success of this approach has been demonstrated by the genetic and molecular dissection of the pathways that set up the Drosophila body pattern (Lee et al., 1989). In the meantime, mutagenesis screens have also been carried out successfully in mice (Balling, 2001; Brown and Balling, 2001; Brown and Hardisty, 2003; Hrabé de Angelis et al., 2000; Nolan et al., 2000). A well-known example of this technique, emphasising the power of the phenotype-driven approach, was given by the identification of the Clock gene based on studies performed in the mid-1990s (Vitaterna et al., 1994; Antoch et al., 1997). These findings gained fundamental insights into the molecular mechanisms underlying mammalian circadian rhythms.

2. The phenotype-driven approach

As outlined above, complementary to the ‘gene-driven’ analysis of gene function such as studies in knockout mice or transgenics (e.g., Müller and Keck, 2002), ‘phenotype-driven’ approaches are currently performed and may be equally important. In search of mouse mutants modelling psychiatric disorders, we have to face the problem that the clear minority of such disorders are caused by single gene defects. An extremely rare example of such a direct influence is the so-called Brunner

syndrome: Here, a discrete genetic defect leads to a loss of the production of the monoaminooxidase A protein (Brunner et al., 1993). In contrast, most of the common psychiatric disorders are characterised by a combination of symptoms, which can be separated into so-called endophenotypes. The term "endophenotype" refers to a set of behavioural and/or physiological characteristics accompanying a basic process that is altered in relation to the illness being studied (Freedman et al., 1999). At the behavioural level, for example, depression is associated with anxiety and autism is accompanied by deficits in social interaction. Although it is unrealistic to screen for mouse models that display the whole complexity of behavioural alterations characterising a psychiatric disorder, specific behavioural traits resembling symptoms or endophenotypes of these disorders can readily be modelled in mice (Holsboer, 1997; Müller and Keck, 2002; Picciotto, 1999). It is, therefore, important to note that this more narrowly defined endophenotype approach does not necessarily have to capture specific symptoms which are part of the clinical diagnosis, but rather may focus on a core and basic process or function which is abnormal in the clinical population under study and that is thought to be related to the manifestation of the disorder. This approach may also make screening for genetic abnormalities associated with the disorder more successful, because the genetic factors associated with a very discrete process (which could be mediated by a small number of genes) rather than the entire syndrome (which is likely to be caused by a complex set of interactions between multiple genes) are being studied (Freedman et al., 1999; Bakshi and Kalin, 2002).

An important criterion for developing animal models to study psychopathology involves establishing the validity of the model as a true representation of the process of interest (Geyer and Markou, 2002). Despite the availability of a long list of approximately 1000 mouse mutants (Lyon et al., 1996), there remains an appreciable lack of behavioural phenotypes in mouse models enabling us to learn more about the aetiology of human psychiatric disorders. This lack may be compensated by phenotype-driven ENU-mutagenesis programs, which aim at identifying novel phenotypes without any a priori assumptions (Brown, 1998; Hunter et al., 2000; Rossant and McKerlie, 2001).

2.1. Dominant and recessive screens

The strategies used in ENU-programs include both dominant and recessive screens. In any case, male mice are treated with ENU and mated with nontreated females. Each F1 individual of the resulting offspring is genetically unique and screened for phenotypic alterations. Test breeding of selected F1 animals then confirms the genetic nature of its phenotype, which can be recovered by outcrossing. From the two large-scale ENU-screens performed in England (Harwell; http://www.mgu.har.mrc.ac.uk/mutabase/) and Germany (GSF Research Centre, Neuherberg; http://www.gsf.de/isg/groups/neu-mouse.html), it is known that about 2% of the F1 animals showed phenotypic alterations which could be proven to be heritable (Hrabé de Angelis et al., 2000; Nolan et al., 2000).

Being interested in human disorders, which are genetically regulated by a recessive mode of inheritance, recessive ENU-screens have also been carried out. In this case, the logistic of breeding protocols is more difficult, as F1 animals (now called G1) are again mated with wildtype mice and the resulting G2 females are back-crossed with their fathers. This breeding regime finally results in an offspring from which 25% will be homozygous for a potential mutation (Balling, 2001).

The last step in ENU-mutagenesis involves mapping of the mutation by using polymorphic markers at approximately 10-cM intervals, followed by a candidate gene approach (for detailed description see Beckers and Hrabé de Angelis, 2002).

The power of ENU-mouse mutagenesis is due to the advantage of hypothesis-free phenotyping. At the same time, this advantage represents the major challenge in large-scale mutagenesis programs as screening assays have to be quick, easy to perform, and as detailed as possible. The power of mouse mutagenesis, therefore, critically depends on the phenotyping procedures performed.

3. Concepts in behavioural phenotyping

To make full use of the phenotype-driven approaches, interdisciplinary cooperation is vital, covering the complexity in phenotypic analysis of the whole organism to the molecular level. Behavioural phenotyping in ENU-mutants is especially challenging, as behavioural profiles have to be identified in single individuals. For high-throughput screening, approaches have been made to establish standardised screening protocols including a combination of well-validated, easy to perform behavioural tests (Crawley and Paylor, 1997; Rogers et al., 1999).

3.1. The unidimensional approach

Various test paradigms exist to assess behavioural parameters in rodents. These tests are valuable tools in determining the implication of genetic factors in the whole complexity of behaviour, which has extensively been validated, for example, by pharmacological studies. It should be taken into account, however, that behavioural expressions represent a combination of behavioural dimensions influenced by genetic as well as environmental factors. The results of behavioural tests might be strongly influenced by testing conditions and the test procedure used. It is, therefore, essential to carefully define these factors when phenotyping behaviour in animals.

Among the most frequently used paradigms are tests for unconditioned behaviour (Crawley, 1999). In these tests, rodents usually are confronted with a novel environment or stimulus and behavioural patterns being indicative for anxiety, locomotion, or exploration, are monitored.

The open field test (Hall, 1936), for example, is regularly used as test for locomotor activity but also to assess anxiety-related behaviour (Clement et al., 1997; Crabbe, 1986; Flint et al., 1995). Probably the most frequently used test for unconditioned behaviour is the elevated plus maze that was first introduced by Pellow et al. (1985). The elevated plus maze is based on the observation that rodents tend to avoid elevated areas (Montgomery, 1958) and avoidance of the open arms is interpreted as anxiety (Lister, 1990; Pellow et al., 1985; Rodgers et al., 1997). Moreover, it has been argued that the elevated plus maze also allows to control for locomotor activity (Belzung and Griebel, 2001; Hogg, 1996; Rodgers et al., 1992). The reliability and sensitivity of this test are increased by using more detailed approaches to analyse rodent behaviour on the elevated plus maze, like including, for example, risk assessment behaviour (Cruz et al., 1994; Griebel et al., 1993; Rodgers and Johnson, 1995; Rodgers et al., 1997).

Stress-coping behaviour usually is assessed by the so-called forced swim test, which has been established by Porsolt et al. (1977) as a behavioural paradigm to identify compounds with antidepressant efficacy in humans: Mice are forced to swim in a glass cylinder filled with water, thus preventing the animal to escape. During the first exposure, the animal is thought to learn that it cannot escape the situation and, consequently, in subsequent tests the time the animal spends immobile is increased. However, it remains an ongoing matter of discussion, whether or not an increase in escape-oriented behaviour may rather be secondary to changes in cognitive performance (Montkowski et al., 1995; De Pablo et al., 1989) or to anxiety-related behaviour (Ferre et al., 1994).

The water maze or the radial arm maze are often used to evaluate cognitive performance in mice (Hodges, 1996). Both tests are focused on spatial learning and memory capacities in mice and have extensively been used in genetically engineered mice (Crawley et al., 1997; Rawlins and Deacon, 1993). Fear-related memory processes are investigated by avoidance tests, frequently representing foot-shock paradigms.

3.1.1. Caveats of the unidimensional approach

Despite their undisputed use, most of the test procedures described above are predictive of only a small spectrum of behavioural patterns. Consequently, a series of tests, i.e., a multiple test battery, is necessary to obtain a behavioural profile (Escorihuela et al., 1999; Rogers et al., 1999). However, testing the same animal in a multiple test battery is likely to induce interferences between distinct tests (Belzung and Le Pape, 1994). For example, repeated testing can lead to habituation or sensitisation, respectively, processes which are affected by cognitive abilities. Therefore, testing of naive animals usually is postulated for initial phenotyping procedures. Concerning ENU-mutants, however, this procedure is impossible, as single individuals have to be characterised. In addition, ENU-mutagenised mice routinely are group-housed for logistic reasons. As a transfer to a test environment accompanies the above-mentioned tests, a multiple test battery implies repeated periods of social isolation for each individual. As the latter is a well-known stress-factor in mice (Ohl et al., 2001a; Von Frijtag et al., 2000), it is likely that behavioural performance in subsequent tests is confounded by the effects of repeated social isolation.

Finally, multiple test batteries of behavioural paradigms are extremely time consuming and, consequently, not feasible for high-throughput screening. Screening protocols such as the SHIRPA-protocol (Rogers et al., 1999) list most behavioural tests which are used for the identification of animal models for psychiatric disorders, in the tertiary screen only, i.e., not within the high-throughput screen.

3.2. The multidimensional approach

To effectively screen for behavioural alterations in a high-throughput manner, different approaches are required which allow for the implementation of the ethological relevance of behaviour. The minimum requirement for such a test in rodents must allow the animal to display its natural behavioural repertoire. It is also important to take into consideration the potential interaction of behavioural dimensions like, for example, exploration, locomotor activity, or cognitive processes, as these dimensions may strongly confound each other. Thus, more complex, observation-based analyses should be performed with respect to the rich behavioural repertoire displayed by rodents (Belzung, 1999; Rodgers et al., 1997; Spruijt et al., 2001).

3.2.1. The modified hole board test

Studies based on behavioural tests, which are focussed on a more detailed ethological analysis of experimental animals in a single complex paradigm, may overcome the disadvantages mentioned for unidimensional approaches (Cruz et al., 1994; Lister, 1990; Rodgers et al., 1997; Wilson, 2000). The modified hole board test is based on the concept that the rich behavioural repertoire of rodents can only be displayed in an adequate, i.e., rich, testing environment. The test essentially comprises the characteristics of a hole board (File and Wardill, 1975; Lister, 1990) and an open field test. In the modified hole board set-up, a hole board, with all holes being covered by a movable lid, is placed in the middle of a box, thus representing the central area of an open field. The experimental box is enlarged by an additional compartment where the group mates of the experimental animal are placed during the test

period, being separated from the test area by a transparent partition (Fig. 1). In both rats and mice, it was demonstrated that the modified hole board enables the investigator to detect and dissociate alterations in a wide range of behaviours, including anxiety-related behaviour, risk assessment, exploration strategies, locomotor activity, arousal, social affinity, and cognition (Ohl et al., 2001b,c, 2002, 2003). In contrast to other behavioural tests, the modified hole board, due to the presence of the group mates, avoids isolation stress in experimental animals during testing, a factor which is well known to affect behavioural performance (Ohl et al., 2001a). In general, test assays such as the modified hole board enable the animal to display a variety of behavioural patterns which, in combination with the careful analysis by a trained observer, offers the opportunity to also discover subtle and unexpected behavioural effects of genetic alterations.

3.2.2. Behavioural dimensions assessed in the modified hole board test

3.2.2.1. Avoidance behaviour. As known from both field studies and laboratory observations, rodents tend to avoid the unprotected area of a novel environment when first entering it (Barnett, 1963; Belzung and Le Pape, 1994; Treit and Fundytus, 1989). ENU-mice, which display behavioural alterations of their avoidance behaviour, may represent an interesting model for anxiety.

Notably, the expression of avoidance behaviour depends on the visual capabilities of the animal and can further be influenced by its locomotor activity, motivational factors, and also by its exploration strategy. Therefore, it is mandatory to simultaneously control for these dimensions to obtain a reliable behavioural profile.

3.2.2.2. Exploration. Being confronted with novelty, behaviour in rodents is determined by the conflict between the drive to explore unknown areas/objects and the motivation to avoid potential danger (approach-avoidance conflict). Exploration behaviour summarises a broad spectrum of behavioural patterns such as risk assessment behaviours, walking, rearing, climbing, sniffing, and manipulating objects (Barnett, 1963; Kelley, 1993; Sheldon, 1968). It is suggested that exploration is gradually inhibited by anxiety, and, therefore, might represent an indirect measurement of anxiety (Crawley and Goodwin, 1980; Handley and Mithani, 1984; Pellow et al., 1985). The inhibition of exploration behaviour can be reversed by anxiolytic compounds (Belzung and Berton, 1997; Griebel et al., 1993; Rodgers et al., 1992) but primary alterations in exploratory motivation may confound measures of anxiety (Belzung, 1999), which has to be taken into account when behaviourally phenotyping mice.

This is nicely demonstrated by the following example: As described above, a mouse or rat will typically start to walk around the walls of an unknown area (thigmotaxis), this being one strategy but, notably, different exploration strategies exist in rodents (Golani et al., 1999; Ohl et al., 2001b). Using a "home base" building strategy, some inbred mouse strains first explore the close surroundings of their starting point instead of walking around the area (Ohl et al., 2001b). As a consequence, standard parameters that use avoidance behaviour will not detect anxiety while alterations in the exploratory strategy will indicate anxiolytic effects. Based on observations from the modified hole board test, both directed and general exploration strategies can be identified and taken into account for interpretation of the data.

3.2.2.3. Risk assessment. When confronted with a threatening stimulus, rodents display species-specific behavioural patterns, such as stretched attends and directed sniffing, which are categorised as risk assessment behaviour (Blanchard and Blanchard, 1989; Cruz et al., 1994; Rodgers et al., 1997). The biological function of these behaviours is to gather information regarding the potential threat by cau-

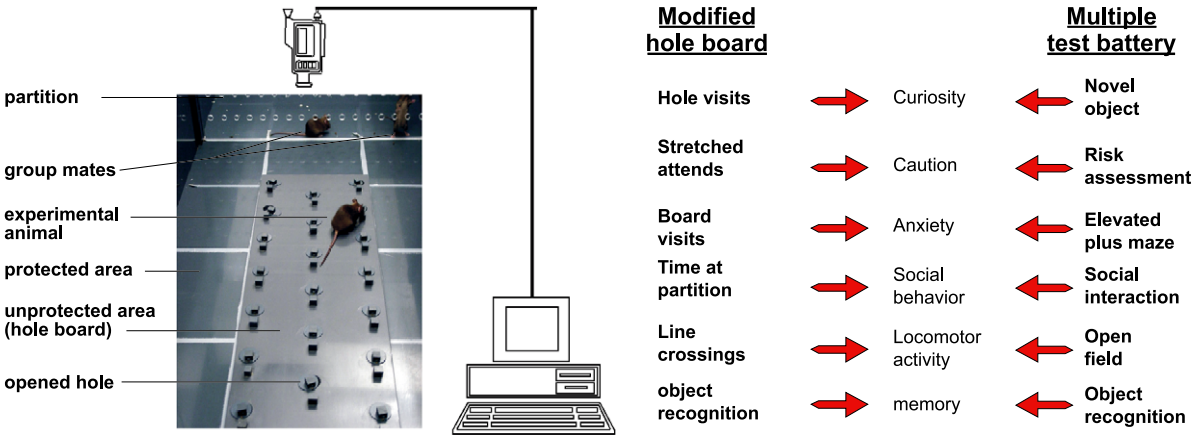

Fig. 1. Comprehensive behavioural analysis based on the modified hole board test enables to differentially evaluate the behavioural profile in mice and, thus, allows for replacement of a time-consuming multiple test battery.

tiously approaching the threatening stimulus or by scanning the surroundings. Risk assessment behaviour is considered to be a defense behaviour (Blanchard et al., 1993). It is of note that factor analyses on complex ethological measures found risk assessment behaviour to represent a behavioural dimension independent from avoidance behaviour (Cruz et al., 1994; Ohl et al., 2001c; Rodgers and Johnson, 1995).

In recent years, extensive studies have been performed, especially by Caroline and Robert Blanchard and colleagues, (i) to evaluate whether human and rodent defensive behaviour in response to threat show parallels (Blanchard et al., 2001b) and (ii) to characterise the predictive validity of specific defense patterns in rodents for anxiety-modulating compounds (for review, see Blanchard et al., 1997). They could show that distinct defense patterns such as defensive threat or risk assessment are highly sensitive for drugs known to be effective in the treatment of generalised anxiety disorder (Blanchard et al., 2001a; Graeff, 2002). Consequently, these behavioural patterns should be carefully evaluated when searching for novel animal models of anxiety disorders.

3.2.2.4. Social affinity. ENU-mice are routinely group-housed in groups of five or six individuals. As the set-up of the modified hole board test allows the presence of the group mates during testing, social affinity can be assessed for each individual. This parameter is known to be altered in psychiatric disorders such as depression and schizophrenia (Seong et al., 2002).

Importantly, the presence of the group mates allows for avoiding social isolation of the experimental animal during testing, a factor which is well known to strongly confound the behaviour of group-housed mice.

3.2.2.5. Cognition. Since a multitude of psychiatric disorders are accompanied by cognitive dysfunctions, it would be of great advantage to be able to identify cognitive alterations already during initial phenotyping in the modified hole board. We, therefore, integrated an object recognition test into the modified hole board set-up. The performance of this task is known to be impaired in patients suffering from Alzheimer's disease or amnesia (Caterini et al., 2002; Doninger et al., 2001). In validation studies, initial phenotyping could be reproduced in the selective object recognition test, underlining that the modified hole board test is a reliable large-scale screen for cognitive alterations.

3.2.3. Selecting specific or complex behavioural alterations

As the modified hole board offers the possibility to evaluate a variety of behavioural patterns, one can either screen for selective behavioural alterations or for more complex combinations of behavioural characteristics. In any case, exact selection as well as exclusion criteria can be defined.

In search for monogenic behavioural traits, the primary approach is the selection of those mice, which are distinctly altered in terms of one behavioural dimension. Each behavioural dimension is indicated by several parameters and as selection criterion, one could define that all these parameters have to be altered while possible confounding factors, such as locomotor activity, have to be inconspicuous and would otherwise represent an exclusion criterion.

However, point mutations may also affect one integrated factor of a complex pathway, possibly resulting in more multi-layered behavioural alterations. Therefore, it may also be worthwhile to define more complex behavioural traits to screen for. As an example, it is known that depressive patients often display reduced motivation, increased anxiety, and reduced social interest. A mouse showing this specific behavioural profile would thus be an interesting animal model for distinct core features of human depression.

3.2.4. A screening schedule for ENU-mutants

The selection of a mutagenised mouse as a potential mutant of interest results in a time- and money-consuming program, ranging from test breeding to gene mapping. Therefore, the confirmation of behavioural alterations found in the primary screen is indispensable. Following the multidimensional approach, repeated testing of mice in different tests is not necessary to obtain a precise behavioural profile. This enables the investigator to confirm behavioural alterations by repeating the modified hole board test after several days of undisturbed recovery. Extensive pilot studies performed in our laboratories in different mice strains have proven that the behavioural strategy displayed during the first test can reliably be found also in the second test. Finally, to exclude context-specificity of the identified behavioural characteristic as well, a selective test, being indicative for the identified behavioural trait of interest, can be performed (Fig. 2).

3.2.5. Expected outcome

Up to now, behavioural phenotypes identified and confirmed in ENU-mutants are restricted to more neurological defects, resulting in head tossing, circling or sensory alterations (Balling, 2001; Sayah et al., 2000). Despite the fact that several high-throughput screens for behaviour in mice have been described (Tarantino et al., 2000; Rogers et al., 1999), novel mouse mutants for common psychiatric diseases have not been published from an ENU-screen as yet. This may also be due to the fact that hierarchical behavioural screening in single individuals, which is the present state of the art, is likely to identify behavioural alterations which mainly represent the ability to cope with repeated testing. One famous exception is the mutant called Clock which shows alterations in the circadian rhythm, thus enabling to gain fundamental insights

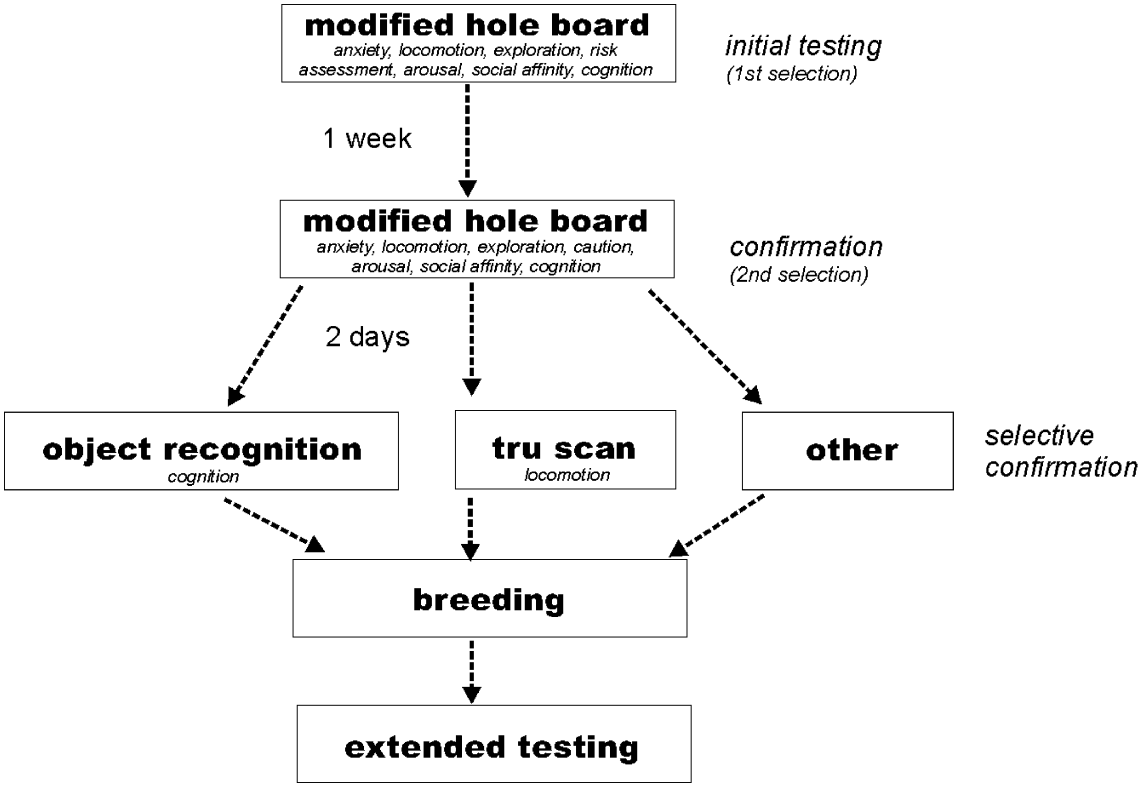

Fig. 2. Behavioural screening schedule for ENU-mutagenised mice. Both initial and confirmation testing are done by use of the modified hole board. To exclude context-specific behavioural alterations, selective tests are performed in addition.

into the molecular mechanisms underlying mammalian circadian rhythms (King et al., 1997; Vitaterna et al., 1994).

Using a large-scale screening procedure based on the modified hole board test in mice may be more promising since it is based on complex behavioural data obtained from one single test. First behavioural screenings in ENU-mutagenesis mice demonstrated that it is possible to identify and dissociate mutants with alterations in distinct behavioural dimensions. Moreover, an appreciable number of animals selected from the 1st test can be confirmed in the 2nd test (Fig. 3). Although the genetic nature of their behavioural characteristics can only be proven in a relatively small percentage of ENU-mice, this finally can result in the establishment of a large panel of novel mouse models of psychiatric disorders, given the large-scale nature of the ENU-mutagenesis program.

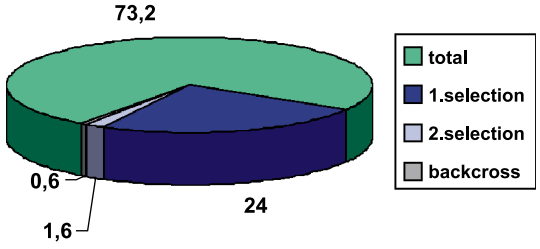

Fig. 3. Calculated from the total number of F1 animals screened during 24 months at the Max Planck Institute of Psychiatry, 24% of the animals showed behavioural alterations which could be confirmed in 1.6%. To date, 0.6% of the initially screened animals are in the backcross-status.

4. Outlook

4.1. Objectivity based on automatisation

During recent years, some tendency towards automatisation of behavioural tests has established itself in order to standardise measurements and to avoid subjective interpretation of the animals' behaviour. As only very few behavioural patterns can be recorded automatically, this increase in standardisation causes a lack of sensitivity, because more subtle behavioural alterations, such as changes in risk assessment behaviour, remain undiscovered. Moreover, automatic recordings might not be able to detect yet unknown behavioural characteristics, which are of major interest in behavioural screening of ENU-mutants, and alterations in strain-specific exploratory strategies are likely to produce wrong results when analysed by use of predefined parameters (Ohl et al., 2001b). Thus, automatisation of behavioural analyses may only be of limited use when searching for unknown behavioural phenotypes.

4.2. Methodological development

Behavioural tests performed in a specific test arena remain artificial to a certain extent, as they are restricted to situation-evoked behaviour, thus, excluding the evaluation of baseline behaviour and the analysis of behavioural long-term effects. The optimal test environment is one that reduces experimental confounds while increasing throughput and efficiency. This could be done by monitoring an animal in its home cage and over a longer period of time, i.e., hours or even days. As it has been lined out for ethological testing in the modified hole board, comprehensive analysis of data obtained from the home cage also takes into consideration that behavioural and physiological dimensions in a given individual are depending on each other, and it enables the investigator to analyse the interaction of those dimensions. However, to date, home cage observations and especially the analysis of the resulting amount of data is extremely time-consuming and, therefore, not suitable for high-throughput screening.

4.3. Novel animal models

The definition and use of endophenotypes in animal models of psychiatric illness is a developing area. By use of ENU-mutagenesis, some promising candidates are currently under investigation. This approach, along with the recent completion of the Human Genome Project, holds great promise for the identification of completely novel genes in psychiatric disorders beyond the usual suspects such as those involved in monoaminergic system regulation. Pharmacologic studies on these genetically altered animals will then be important in exploring potential treatments. Using these methods, it may be possible in the near future to further increase rapid crosstalk between animal studies and clinical findings.

Moreover, there is raising evidence that behavioural testing in preclinical animal models represents a bottleneck in psychotropic drug discovery. Nevertheless, such investigations in preclinical models of human psychopathology are required, for example, to provide initial assessments of the functional effects of novel compounds in the integrated organism. To date, due to the limited investment in their development over the past decades, there are only few animal models as well as behavioural paradigms, such as the tail suspension and forced swim tests for antidepressants and the prepulse inhibition test for antipsychotics, that are of clinical relevance (e.g., Dalvi and Lucki, 1999). However, knowledge about the neurobiological changes underlying these behavioural paradigms is very limited (e.g., Keck et al., 2001, 2002, 2003). Paradigms that are more laborious are, therefore, necessary to identify new therapeutic targets through the investigation of the interacting systems that contribute to the disorders' symptomatology or the therapeutic effects of established drug treatments. This may ultimately help to increase our rather limited knowledge about the neurobiology of psychiatric disorders when compared to the great advances in other fields of medicine (Andreasen, 1997; Geyer and Markou, 2002).

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