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Research report

Performance of F2 B6x129 hybrid mice in the Morris water maze, latent inhibition and prepulse inhibition paradigms: Comparison with C57Bl/6J and 129sv inbred mice

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Abstract

Assessment of cognition and information processing in mice is an important tool in preclinical research that focuses on the development of cognitive enhancing drugs. Analysis of transgenic (TG) and knockout (KO) mice is usually performed on a F2 B6x129 background. In the present study, we have compared performance of F2 B6x129 hybrid mice (F2 mice) with that of the two parental inbred strains (C57Bl/6J and 129sv mice), and a wild-type (WT) strain (with a combined B6x129 background) in three cognitive/information processing paradigms.

It was found that the F2 mice outperformed either of the parental strains and provide a control sample with good baseline performance in the Morris water maze (MWM). Reliable deficits could be obtained in learning and memory in this paradigm following injections with scopolamine (0.16 mg/kg) in the F2 mice, which can potentially be used to test effects of reference and novel compounds in order to develop cognitive enhancing drugs. Furthermore, it was shown that the four genotypes showed normal latent inhibition (LI) using the conditioned taste aversion (CTA) paradigm and exhibited no differences in prepulse inhibition (PPI) levels.

Following the setup of these procedures in mice, we are now able to compare the effects of gene knockout/mutations used for target validation with results in the present study as a frame of reference.

Keywords: Morris water maze (MWM); Latent inhibition (LI); Conditioned taste aversion (CTA); Prepulse inhibition (PPI); Locomotor activity (LMA); Scopolamine; Mice genotypes

1. General introduction

Cognitive and information processing deficits are a core feature of schizophrenia [4,34,68]. Antipsychotic naïve patients, compared to normal controls, have been shown to possess generalized impairment that was most marked for tests of abstraction, attention, verbal memory, spatial memory and language abilities [11,29]. These deficits have also been considered to be responsible for long-term disability in schizophrenia [13,63]. Besides (early) environmental factors, also genes play an important role in deficits that characterize this disorder.

The availability of embryonic stem (ES) cells for gene-targeting has resulted in laboratory mice becoming important animal models of human psychiatric diseases, such as schizophrenia. Therefore, assessment of cognition and information processing in mice is an important tool in preclinical research that focuses on the development of cognition-enhancing drugs.

In gene-targeting research, after successful isolation of ES cells that contain the desired mutation, the cells are injected into blastocysts from a donor strain to produce chimeras, which themselves can be mated to another strain that is a good breeder, most often C57Bl/6 (B6) mice. However, this introduction of another genome brings with it a particular confounding effect of genetic background: the so-called "flanking gene problem" or genetic bias resulting from genetic linkage between the targeted locus and neighboring genes. Even if the latter mating strategy is used, and the mutation is backcrossed to another strain for several generations, attention should still be paid to the ES cell line source strain, most often 129 mice. It is the source of alleles co-inherited with the mutation that may affect

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the behavioral phenotype of the tested mice, possibly leading to false positive or negative results. Inbred strains of mice differ in many behavioral phenotypes, so that the same gene mutation can appear to have different phenotypic effects when introduced onto different genetic backgrounds [12]. Without characterizing the background lines, it is difficult to conclude that observed phenotypes in knockout mice are due to a specific mutation rather than to genetic contributions from one of its parental lines. So, the possibility has been raised that behavioral abnormalities seen in null-mutant mice might be determined by their genetic background rather than by loss of gene function, especially when the 129 mouse strain is used as supplier for ES cells.

Many studies have been performed in inbred strains. However, the analysis of transgenic (TG) and knockout (KO) mice is usually performed on a F1 hybrid genetic background or in F2 B6x129 crosses, which have been shown to provide a suitable genetic background. Advantages of using F1 hybrid mice include genetic and phenotypic uniformity and hybrid vigor. Like the targeted mice, the genetic background composition of the F2 mice, however, varies among littermates because of gene segregation and assortment between all the different loci of the parents from the F1 hybrid parents. They contain exclusively genes derived from B6 or 129 genetic backgrounds [22].

With the purpose to test TG/KO mice in cognitive and information processing paradigms, the main aim in this study was to characterize performance of the F2 B6x129 hybrid genotype (F2 mice) in several tests: the Morris water maze, latent inhibition and prepulse inhibition. Results were compared with those of the two parental inbred strains (C57Bl/6J or B6; 129sv or 129 mice). Also, a wild-type strain from Lexicon Genetics Inc. (with a combined B6x129 background) as a representative of WT mice of multiple different knockout mouse strains was included. The three separate experiments for each test will be presented here in three subsections. Additionally, spontaneous locomotion was measured in the four genotypes to control for differences in general activity levels. These data will provide a frame of reference for subsequent studies in TG/KO mice in these paradigms.

2. Morris water maze experiments

2.1. Introduction

The Morris water maze is a spatial navigation task that is used to measure spatial learning and memory capability in laboratory animals. Mice are required to swim in a pool of water until they locate a submerged platform using visual cues placed around the test room. Evidence that spatial memory has formed after repeated training to a given platform location is derived from a probe trial in which the platform is removed. Continued searching in the former platform location provides measures of spatial retention.

It has been shown that performance in the water maze varies across strains of mice [12,15,28,48,65,71,72,73]. Interpretations as to the impact of single gene mutations for polygenic behaviors like learning and memory will depend in part on the genetic background of the animals used for these manipulations. For example, Steinberger et al. [64] have mapped genetic effects that contribute to the difference between two strains, DBA/2 and C57Bl/6J, using an F2 intercross and methods to detect quantitative trait loci (QTL). They have found two QTL, one on chromosome 4 and one on chromosome 12, that influence behavior in the probe trial of the water maze. Likewise, Owen et al. [48] have tested 12 inbred strains and seven different F1 hybrids on multiple behavioral tasks, including the Morris water maze. They have shown that F1 hybrids performed better in complex learning tasks than inbreds. Therefore, they have concluded that the behavioral performance of F1 hybrids cannot be predicted by simply knowing the learning performance of the two parental strains. Furthermore, individuals in the F2 population, used as genetic background for TG/KO mice, are not genetically identical and new combinations of genes in these individuals may lead to poor learning performance. Moreover, because learning is a polygenic trait, large numbers of individuals from a number of F2 litters must be tested to account for this genetic segregation [71].

In the present study, results in the F2 mice were compared with performance in the B6 and 129 inbred mice and the WT strain. Besides our goal to use these data as information for subsequent experiments in TG/KO mice, we also intended to use the F2 animals for our in house studies in which we induced water maze deficits with amnestic drugs, such as scopolamine. Emerging research supports a role for the muscarinic cholinergic system in schizophrenia [9,26] and the muscarinic antagonist scopolamine has also been shown to impair acquisition and retention in the Morris water maze [27,37,50,56,55]. So, scopolamine was used in these studies to induce learning and memory deficits in the F2 mice, in order to try to ameliorate these deficits with new compounds. Therefore, in the present study, parameter values were analyzed from 100 solvent and 60 scopolamine-treated (0.16 mg/kg, SC) F2 mice from various drug studies. Results will be presented on these in house data to establish whether the F2 mice could also be a suitable genotype for our drug studies with scopolamine-induced deficits. In addition, effects of injections with different solvents in these drug studies were evaluated. A large proportion of recently discovered drugs are very lipophilic molecules with low and variable bioavailabilities. Many approaches have been made to overcome this problem, e.g. including complexation of the drug into cyclodextrins [40,52]. Here, it was our goal to find out whether this additional stressful event would influence performance.

2.2. Materials and methods

2.2.1. Animals

Four mice genotypes (male mice, 10–12-week-old and approximately 25 g in weight) were compared. The two inbred strains were obtained from Charles River (Lyon, France): C57Bl/6J (B6) and 129sv (129) mice. The F2 B6x129 mice (F2) originating from both inbred strains were obtained from the breeding colony at the internal Transgenic Animal Facility (Johnson & Johnson, Beerse, Belgium). A wild-type strain from Lexicon Genetics Inc. was used as a representative of WT mice of multiple different knockout strains. 129sv/EVbrd(LEX1) embryonic stem (ES) cells were used. Targeted ES cell clones were injected into C57Bl/6 blastocysts, and the resulting chimeras were mated to C57Bl/6 females to generate animals heterozygote (±) for the mutation. These were subsequently crossed to generate all three genotypes. For the present studies, only

the WT mice were included for analysis and compared with above-mentioned three mice genotypes.

All animals were housed in their individual home cages 3 days prior to testing under controlled conditions (temperature: 23 °C, humidity: 60%, normal light–dark cycle: light on 06:00 until 18:00 h). Animals were provided with a supply of food and water ad libitum. All efforts were made to minimize animal discomfort and for limiting the numbers of animals used. The local Johnson & Johnson Ethical Committee approved all experimental protocols and the actual experiments were carried out following the procedure described by the guidelines of the European Community Council Directive of November 24th, 1986 (Declaration of Helsinki 86/609/EEC).

Above-mentioned four mice genotypes were compared in the Morris water maze paradigm to measure effects on spatial acquisition and retention: B6, 129, F2 and WT male mice. Each experimental group consisted of 10 animals. In the second part of the study, parameter values were analyzed from 100 solvent and 60 scopolamine-treated F2 mice from the drug studies. In these experiments, the animals were injected subcutaneously (SC) with a variety of solvents and with scopolamine, each day prior to swim sessions.

2.2.2. Apparatus

The Morris water maze paradigm was used to assess spatial learning and long-term memory. In this paradigm, mice are trained to locate a submerged platform, using extra-maze visual information. The apparatus consisted of a grey polyethylene circular pool (120 cm diameter, 50 cm deep) filled with water (water temperature 21–22 °C, water level 35 cm high). A transparent plexiglas escape platform (10 cm diameter) was placed in one of the four quadrants of the pool, its surface 0.5 cm below the surface of the water. Numerous, constant, visual cues surrounded the tank to facilitate orientation. A computerized tracking system and image analyzer (EthoVision® 3.0.15, Noldus, Wageningen, The Netherlands) was used to monitor swim patterns. The camera hung perpendicular to the center of the pool. The image analyzer tracked the center of each mouse with a sampling rate of 25 Hz, and allowed the calculation of escape latency (in s), distance (in cm), velocity (in cm/s), time spent in different zones of the arena (in %) and frequency of visits in a certain area.

2.2.3. Procedures

The protocol according to De Bruin et al. [19] was adjusted for mice. Three days prior to the actual test, the mice received three pre-training trials in a small container (30 cm × 10 cm), filled with water at room temperature to a depth of 5 cm. In this case, a small white polystyrene block was left to float in the container and the aim for the animal was to learn how to climb onto and balance on the platform, and in general to habituate to perform a task in water. This short adaptation process was undertaken, because it is thought that the immersion of the mice into the water, and the initial sensation of being trapped in it, may cause considerable stress during the first stages of the actual test [16]. Therefore, this procedure attempts to minimize that problem.

For the actual test, the pool was virtually divided in four quadrants (on the computer). The escape platform, positioned in the center of one of the quadrants, remained in a fixed position. Four different starting positions were spaced around the perimeter of the pool (one position per quadrant). Each trial, the animals were put into the water, facing the wall, at randomly assigned starting positions. The acquisition phase of the experiment consisted of a series of 24 training trials, lasting up to 60 s each (6 trials per day for 4 consecutive days, intertrial interval approximately 30 s). Mice failing to find the platform within 60 s were gently placed on the platform and left there for about 15 s to orient. The latency to find the submerged platform, the distance traveled during the trial, swim velocity and time spent in the periphery were registered. Swimming speed and time in periphery were included as parameters, since these can strongly bias the outcome of Morris water-escape results. Especially, when testing compounds, these might specifically affect certain parameters, such as reducing swim velocity due to, e.g. motor problems or sedation.

On the 5th day (trial 25), the platform was removed for the probe trial. Mice were placed in the quadrant opposite to that where the platform was previously located and were allowed to swim in the pool for 90 s. This probe trial was performed approximately 24 h after the final acquisition trial, for each mouse. The first 30 s of the 90 s probe trial was analyzed because it was determined that any preference for the target quadrant will be most pronounced during the first 30 s of the trial, and analysis of the animal's behavior after this time is likely to underestimate the spatial ability of the animal [5]. The percentage of time spent in the quadrant where the platform was previously located (target quadrant) and the number of platform area crossings were used as a measure of spatial memory. The number of crossings can be considered as a measure for accuracy. Again swim velocity was registered. Additionally, also the time spent in the start quadrant was analyzed in order to determine if animals stay longer in this quadrant, because of non-specific behavioral effects.

2.2.4. Drug treatment

In the water maze drug studies, particular solvents (SC, 10 ml/kg injection volume) were administered 15–60 min prior to testing. The following different solvents were used in the drug studies to dissolve some of the pretreatment drugs (saline; H_2O + mannitol; 1% HCl; 5% hydroxypropyl-β-cyclodextrin (CD) + 1% HCl; 10% CD + 1% HCl; 20% CD + 1% HCl; 10% captisol + 0.25% polyvinylpyrrolidone (PVP) + 1% HCl; 20% captisol + 0.25% PVP + 1% HCl). In addition, either saline or scopolamine (0.16 mg/kg, SC) was injected 30 min prior to testing. See Table 1 for the exact treatment groups. (−)-Scopolamine hydrobromide was purchased from Sigma–Aldrich (Bornem, Belgium). The particular dose of scopolamine (0.16 mg/kg) was previously chosen based on an in house dose finding study (unpublished data).

2.2.5. Statistical analyses

MANOVA General Linear Model (GLM)-analysis (SPSS 11.5 Statistical Package) was used to determine the effects of the between subject factor (genotype), the within subject factor (day) and genotype by day interaction on the

Table 1
Morris water maze treatment groups in house data

Treatment-schedule historical in house data	Groups (number of animals in each group)	
	Control	Scopolamine
Treatment solvents		
No injection	10	10
H_2O + mannitol (T-15 min, SC)	10	10
10% CD + 1% HCl (T-60 min, SC)	10	10
20% CD + 1% HCl (T-45 min, SC)	10	10
10% captisol + 0.25% PVP + 1% HCl (T-45 min, SC)	10	10
20% captisol + 0.25% PVP + 1% HCl (T-45 min, SC)	10	10
1% HCl (T-45 min, SC)	10	
5% CD + 1% HCl (T-45 min, SC)	10	
10% CD + 1% HCl (T-45 min, SC)	10	
20% CD + 1% HCl (T-45 min, SC)	10	
Total	100	60

Control (saline, SC, T-30, SC) and 0.16 mg/kg scopolamine-treated (SC, T-30, SC) mice compared (CD: hydroxypropyl-(-cyclodextrin); PVP: polyvinylpyrrolidone).

dependent variables. Analyses were performed on average values of six trials. One-way Anova was used to analyze group differences on separate acquisition days and the effects on probe trial variables. If appropriate, analyses were followed by post hoc tests according to Tukey to determine differences between genotype groups. Also, the number of animals that reached the platform on day 4 of acquisition and the number of animals that crossed the platform ≥1 during the probe trial were determined. Finally, we tested with the one-sample test whether percentage of time in the target quadrant was equal to chance level for each experimental group (chance level of performance would be reflected in approximately 25% of the time spent in the previous target quadrant, since there are four quadrants). Differences were considered as tendencies for $0.05 \leq P \leq 0.1$ and statistically significant for $P < 0.05$.

2.3. Results

See Figs. 1 and 2 for the results. Although, we have analyzed the swimming distance and velocity parameters, we have decided to not present these results, because in the current experiments these values could be considered as redundant. These parameters did not provide additional information, since they correlated with the latency parameter. However, it should be noted that the distance and velocity parameters can provide essential information, for example, in case the motor capabilities are affected.

2.3.1. Spatial acquisition and memory as a function of genotype

2.3.1.1. Acquisition. Latency to platform and time spent in periphery were significantly decreased over the four days of acquisition (respectively, ($F(3,98) = 34.5$, $P = 0.000$) and ($F(3,98) = 39.8$, $P = 0.000$)) (Fig. 1). Overall, genotypes differed in latency to platform ($F(3,36) = 4.3$, $P = 0.011$) and time spent in periphery ($F(3,36) = 4.6$, $P = 0.008$). Latency was significantly lower in the F2 mice in comparison with the remaining genotypes (129 $P = 0.003$; B6 $P = 0.034$, WT $P = 0.005$). Also, percentage periphery time was significantly lower in the F2 mice in comparison with the other genotypes (129 $P = 0.002$; B6 $P = 0.028$, WT $P = 0.005$). Interactions between day and genotype did not reach significance. The number of animals (out of 10 tested mice) that reached the platform on day 4 of acquisition was 10 in B6, 129 and F2 and 8 in WT mice.

2.3.1.2. Probe trial. Genotypes tended to differ in the percentage of time spent in the target quadrant area ($F(3,39) = 2.7$, $P = 0.063$) and significantly differed in the percentage of time spent in the start quadrant ($F(3,39) = 3.8$, $P = 0.018$), but showed no difference in the number of target area crossings ($F(3,39) = 1.3$, $P = 0.276$). Further, 129 mice spent a significantly lower percentage of time in the target quadrant ($P = 0.040$) and a significantly higher percentage of time in the start quadrant ($P = 0.018$) in comparison with the F2 mice. The percentage of time in the target quadrant was significantly higher than chance level in the B6, F2 and WT mice (respectively, $P = 0.043$, $P = 0.001$ and $P = 0.050$). In contrast, in the 129 mice, the percentage failed to be significantly different from 25% ($P = 0.409$). The number of animals with ≥1 platform

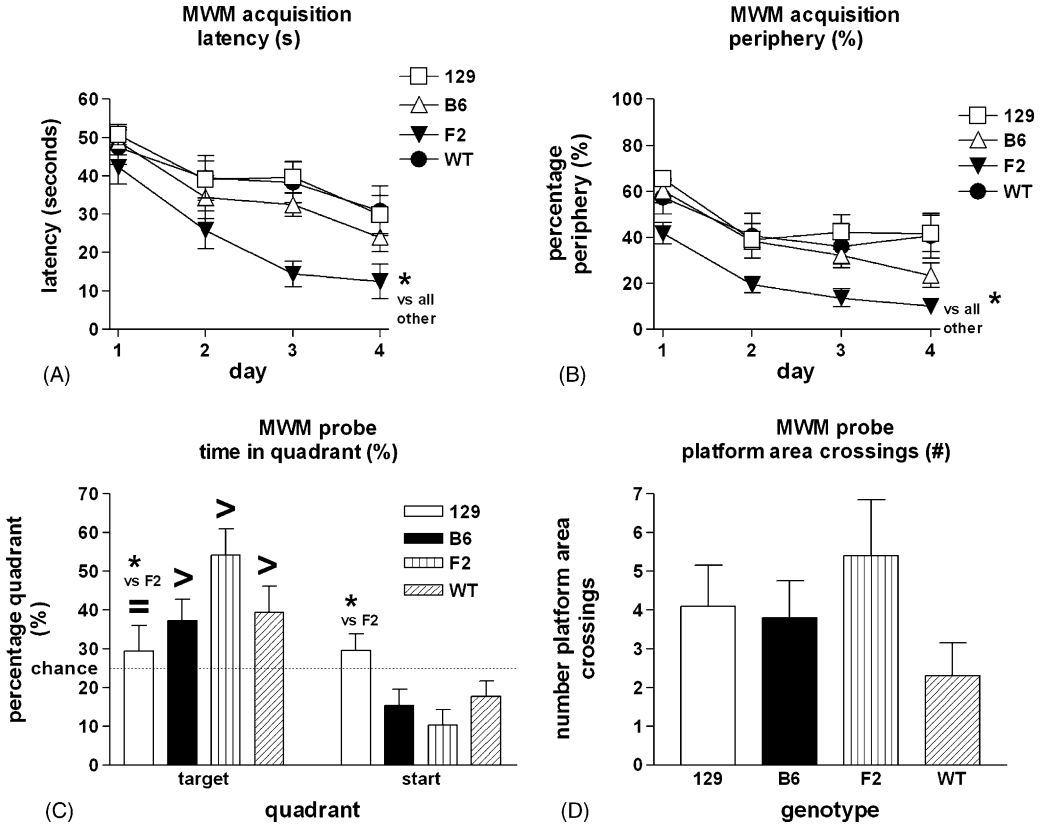

Fig. 1. Morris water maze (MWM) results (mean ± S.E.M.) as a function of genotype. *Acquisition*: latency to platform (in s, A) and percentage of time in periphery (B). *Probe trial*: percentage of time spent in target/start quadrants (C) and number of platform area crossings (D). Asterisk (*) is significant at the 0.05 level (two-tailed); percentage in target quadrant < lower than 25% chance level; (=) equal to 25% chance level; (>) higher than 25% chance level.

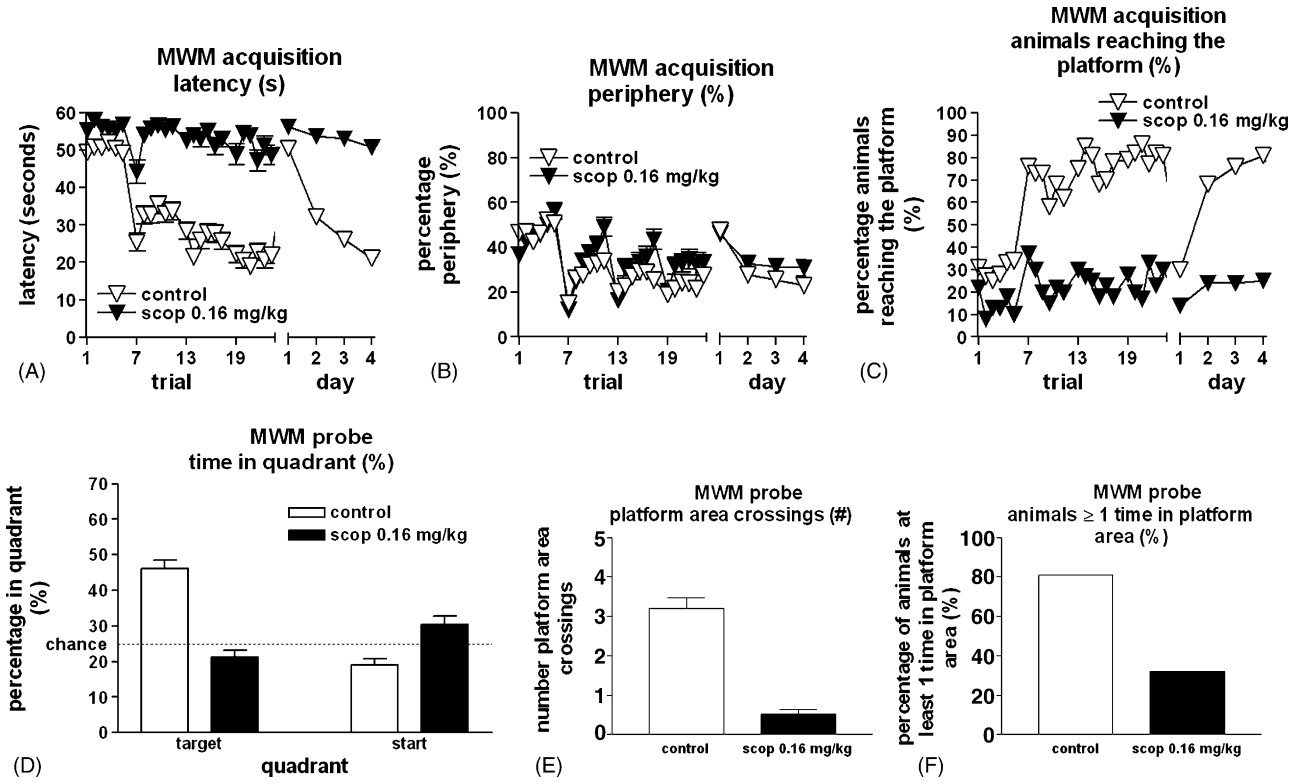

Fig. 2. Morris water maze (MWM) results (mean ± S.E.M.) in 100 solvent and 60 scopolamine-treated (0.16 mg/kg, SC, T-30 min) F2 mice. *Acquisition*: latency to platform (in s, A); percentage of time in periphery (B); percentage of animals reaching the platform on day 4 (C). *Probe trial*: percentage of time in target and start quadrants (A); number of platform area crossings (B); percentage of animals that enter the platform area at least ones (C).

area crossing was 9 in F2, 8 in B6 and 129 and 7 in WT mice.

2.3.2. Spatial acquisition and memory in control and scopolamine-treated F2 mice

In this part of the results section, findings from several drug studies were analyzed: from 100 controls and 60 scopolamine-treated mice (Fig. 2). In all these experiments, control groups treated with different solvents attained normal performance and within each scopolamine group, significant deficits were found in comparison with respective control groups.

2.3.2.1. Acquisition. Latency to platform decreased from 50 to 21 s in control mice (from day 1 to day 4). The percentage of animals reaching the platform increased from 30% on day 1 to 81% on day 4 in this group. In contrast, in the mice that were administered scopolamine, the percentage reaching the platform did not exceed 25%. Latency to platform hardly decreased (from 56 to 51 s). So, the scopolamine-treated mice did not acquire the task. Also, time spent in the periphery decreased from 47% to 23% in control mice. In scopolamine-treated animals, time spent in the periphery was 46% on day 1 and 31% on day 4.

2.3.2.2. Probe trial. Time spent in the target quadrant was above chance level (>25%, 46%) in controls, which was not the case in scopolamine-treated mice (21%). Similarly, the number of platform area crossings was higher in the controls (3.2) than in scopolamine-treated mice (0.5). The percentage of animals crossing the platform area one time or more was 81% in the control group and only 32% in the mice that were administered scopolamine.

2.4. Discussion

First, we have studied genotype differences in performance in the Morris water maze in order to determine which genotype to choose for validation of the model in house and to directly compare results in the parental inbred strains and the F2 generation which has often been used as the background for TG/KO mice. We have found that the F2 mice were superior in task acquisition in comparison with the 129, WT and B6 mice: latency to platform and percentage of time in periphery was lower in the F2. During the probe trial, the F2 performed significantly better than the 129 mice: 129 animals spent less time in the target quadrant (equal to chance level) and remained more time in the start quadrant.

These results are in accordance with those of others [71] who have shown that the F2 and F3 generations of 129xB6 crosses outperform either of the parental strains and they have concluded that these mice provide a control sample with good baseline performance, provided that the samples are large enough to compensate for genetic and epigenetic variability and provided that normal performance in the control group is verified by comparison against a large in house database of mice tested under identical conditions. They have also found that creating congenic lines by backcrossing to an inbred strain is unlikely to

enhance the sensitivity of the Morris maze test and that backcrossing to 129 may even reduce it. As already mentioned in Section 2.1, Owen et al. [48] have tested 12 inbred strains and seven different F1 hybrids on multiple behavioral tasks, including the Morris water maze. They have found that the 129 mice learned the Morris maze task, though at a level inferior to B6 mice, a result that was not confirmed in our study, although they were inferior to the F2 mice. They have shown that nearly 80% of the 129 mice sample exhibited hypoplasia of the corpus callosum (partially or fully disconnected corpus callosum). Thus, 129 mice, in addition to performing poorly in the Morris maze, also displayed aberrant neuroanatomy [39]. So, based on findings in the present study and those of others, we can conclude that above mentioned arguments warrant the decision to use the F2 mice in the water maze validation studies. An interesting other finding in the present study was that the data in the particular Lexicon WT mice were more similar to the data in the 129 mice and did not resemble results in the F2 mice. This could be due to the type of ES cells used by Lexicon (129sv/EVbrd(LEX1)) or might be caused by the particular genetic background composition of the sample of WT mice. This also emphasizes the importance to evaluate results in particular WT mice in each TG/KO study against performance in the F2 mice.

As described in Section 2.1, besides our goal to use data in the current study as information for subsequent experiments in TG/KO mice, we also intended to use the F2 animals for our in house studies in which we induced water maze deficits with scopolamine. Also, effects of injections with different solvents administered during in house drug studies were evaluated to find out whether this additional stressful event would influence performance. It was shown that F2 control groups treated with different solvents attained normal performance and within each scopolamine group significant deficits were found irrespective of solvent pretreatment schedules. So, this means that the particular solvent can indeed be used in future experiments. However, when analyzing the larger group of 100 solvent and 60 scopolamine injected F2 mice from the Morris water maze drug studies, it was apparent that the control animals from the drugs studies were slightly slower in acquiring the task and also performed not as good during the probe trial in comparison with the non-injected F2 mice in the genotype study. The main difference between animals from both studies was that the mice from the drug studies had received daily injections prior to swim sessions. Stress (due to these injections) could have reduced their subsequent performance, which has also been suggested by others [28,35]. For example, in a study by de Quervain et al. [21], rats were trained to swim in a water maze. When the rats were stressed by a mild electrical shock 30 min before being put in the water, they had difficulty locating the platform. In contrast, the researchers have found that if the shock was given 2 min or 4 h before the test, the animals had no difficulties. The impairment in performance at 30 min post-injection was shown to coincide with high blood levels of a glucocorticoid hormone known as corticosterone, which reached a peak about 30 min after the stressful incident. Besides exhibiting hypercorticoid secretion, also pronounced alterations of central norepinephrine and dopamine levels have been reported following stressors [62]. So, the reduction of the performance in the F2 solvent-treated mice versus the non-treated mice from the first genotype study might be due to injection stress and enhanced corticosterone levels. We cannot exclude the roles that the injections with the various solvents (particularly cyclodextrins) might have played in this respect. Nonetheless, the control mice still learned the task very well and the mean percentage of time in the target quadrant during the probe trial was significantly above chance level. In contrast, scopolamine-treated mice showed a clear deficit in both spatial learning and reference memory. The present results show that blocking the central cholinergic system reliably impairs performance in the F2 mice.

3. Latent inhibition experiment

3.1. Introduction

Latent inhibition (LI) is the retardation of associative conditioning resulting from pre-exposure of the conditioned stimulus (CS) alone prior to conditioning. LI has been reported to be disrupted in acutely psychotic schizophrenic patients tested within the first weeks of the current episode of illness or being in an acute phase [2,33,54], while these LI deficits have been shown to be normalized during later episodes, possibly due to the effects of antipsychotic treatment [2,33]. Lubow directly relates LI to the operation of selective attentional processes that are dysfunctional in schizophrenia. In his review, the author refers to the evidence for the involvement of selective attention processes in the acquisition of LI: “Experimental operations that are distraction producing, attenuate LI” [42]. In contrast, Escobar et al. favor a framework in which disruption of latent inhibition and blocking in acute schizophrenics is viewed as an inability to compare and express stored representations (i.e. associative performance deficit) [25].

The LI paradigm as tested in rodents has also been suggested to predict antipsychotic activity, “typical” versus “atypical” action of antipsychotic drugs (APDs) and effectiveness against negative symptoms under different test conditions [61,69]. First, the capacity of drugs to block amphetamine-induced LI disruption has been suggested to predict antipsychotic activity [59,60]. Further, there are certain conditions that do not lead to LI in control rats, for example, when a low number of pre-exposures are presented. Potentiation of LI by APDs administered during conditioning has also been proposed to be an index of antipsychotic activity [69,70]. Secondly, LI can be disrupted in control rats by raising the number of conditioning trials. Interestingly, *N*-methyl-D-aspartate (NMDA) antagonists, such as MK-801 (0.05 mg/kg) and phencyclidine (PCP, 2 mg/kg), administered prior to the conditioning trials, have been shown to induce abnormally persistent LI under these conditions [30,49]. This LI perseveration has been found to be reversed by clozapine (5 mg/kg), but not by haloperidol (0.1 mg/kg), administered during pre-exposure [30]. Therefore, it has been proposed by Weiner and co-workers that perseveration due to NMDA antagonists may model impaired attentional set shifting associated with negative symptoms of schizophrenia.

Strain differences in LI have been reported [14,32]. LI has also been studied in several mutant mice and this has provided information on the involvement of certain genes and receptor systems in LI. For instance, studies in humans and mouse mutants have implicated the coding sequence of the disrupted-in-schizophrenia-1 (DISC-1) gene and the gene encoding neuregulin-1 (Nrg-1) as candidate susceptibility genes for schizophrenia. For example, it has been found that transgenic mice with a disruption of DISC1 protein during development show deficits in latent inhibition [38]. Also, mice heterozygous for a mutation in neuregulin-1's immunoglobulin (Ig)-like domain (Ig-nrg-1 mice) have been found to display behaviors related to a schizophrenia-like phenotype, such as impaired LI [57]. In contrast, latent inhibition has been found to be significantly increased in mice with a deletion of 503 b in the ic3 loop of the M5 muscarinic receptor gene, as compared with controls [67].

In the present study we have used the conditioned taste aversion (CTA) paradigm. This LI procedure involves a pre-exposure phase in which water-deprived animals are allowed access to either water (non-pre-exposed, NPE) or 5% sucrose solution (pre-exposed, PE), followed by a conditioning phase in which animals are allowed access to the sucrose solution and subsequently injected with lithium chloride (LiCl), and a test phase in which animals are allowed access to both the sucrose solution and water. LI is then assessed by comparing the percentage sucrose solution consumed in PE and NPE groups on the test day. CTA is the phenomenon whereby the novel rewarding taste (sucrose solution, CS) is associated with illness (induced by injecting LiCl immediately after the CS) resulting in avoidance of that flavor. LI is demonstrated when PE animals show less aversion as a result of its subsequent association with LiCl than NPE mice. LI parameters were compared in the four genotypes, particularly to establish whether the F2 mice show normal LI in comparison to the other genotypes.

3.2. Materials and methods

3.2.1. Animals

Earlier-mentioned four mice genotypes were compared: B6, 129, F2 and WT male mice. For this experiment, a new batch of animals were tested. Within each group of 20 mice, 10 mice formed the pre-exposed (PE) group and 10 mice formed the non-pre-exposed group.

3.2.2. Apparatus

Drinking bottles of 150 ml were required in this experiment, and were weighed as necessary on a DeltaRange® balance, where 1 g of weight was considered to be the equivalent to 1 ml of drinking solution.

3.2.3. Procedures

The protocol according to Ellenbroek et al. [24] was adjusted for mice. The conditioned taste aversion procedure was used to study latent inhibition. The test duration was 5 days (3 pre-exposure days, 1 conditioning day and 1 test day). Twenty-four hour prior to the start of the pre-exposure phase, the water bottles were removed from the cages of all the animals, so that the animals would be thirsty over subsequent days. On the following 3 days, the NPE group, were given access to plain water, and the PE group were given 5% sucrose solution for 30 min a day. On the conditioning day, all of the animals were given 5% sucrose solution for 30 min, and then immediately after their drinking session, they were given an intraperitoneal (IP) injection of lithium chloride (100 mg/kg) to induce nausea.

On day 5, the test day, all of the animals were given the choice to drink from a bottle containing water or a bottle with 5% sucrose solution. The position of the water/sucrose bottles on the left or right side of the cage was controlled for.

Each day, bottles were weighed before and after the 30 min drinking session, to establish the total liquid consumption (in ml) of each animal. The degree of aversion for the sucrose solution for each group of animals was determined on the test day by calculating the percentage of sucrose solution consumption on day 5 relative to the total fluid intake on day 5.

The objective was to establish whether the animals from the NPE group would learn that drinking of the sucrose solution is associated with becoming nauseated. Therefore, they would be expected to drink less sucrose solution and relatively more water on the test day. This phenomena is called conditioned taste aversion. For the animals that have previously received the sucrose solution on the 3 pre-exposure days (the PE group), it will be more difficult to form the association between the drinking of the sucrose solution and becoming nauseated. They were expected to have learned that the sucrose solution is not a relevant factor in becoming nauseated and show less taste aversion to the sucrose solution. This phenomenon is called latent inhibition. So, we expect PE animals to drink relatively more sucrose solution (of total liquid consumption) or show less aversion for the sucrose solution in comparison with the NPE animals.

3.2.4. Drug treatment

Immediately after their drinking session on the conditioning day, mice were given an intraperitoneal injection of lithium chloride (100 mg/kg, 10 ml/kg injection volume, SigmaUtra LiCl dissolved in saline, L4408, 7447-41-8, Sigma–Aldrich, Bornem, Belgium) to induce nausea.

3.2.5. Statistical analyses

MANOVA General Linear Model (GLM)-analysis (SPSS 11.5 Statistical Package) was used to determine the effects of the between subject factors (pre-exposure and genotype) and pre-exposure by genotype interaction on the dependent variables (liquid consumption on the pre-exposure days PE1-3, conditioning day CSUCROSE, test day TTOTAL and the body weight on the conditioning day CWEIGHT). Effects on the sucrose ratio were analyzed using GLM, and supplemented with additional analyses of covariance (ANCOVA) to test whether concomitant effects of genotype on drinking behavior as such could account for the genotype effects on LI. To this end, ANCOVA of the percent sucrose consumption on the test day were conducted with liquid consumption on the pre-exposure days, the conditioning day, and on the test day as the covariates. If appropriate, analyses were followed by post hoc tests according to Tukey to determine differences between genotype groups. Additionally, the number of NPE animals with a sucrose ratio lower than 50% and the number of PE animals with a sucrose ratio higher than 50% were determined. Finally, we tested with the one-sample test whether the sucrose ratio was equal to chance level for each experimental group (chance level of performance would be reflected in approximately 50% of total liquid consumption on the test day since they could chose between two drinking bottles). Differences were considered as tendencies for $0.05 \leq P \leq 0.1$ and statistically significant for $P < 0.05$.

3.3. Results

See Table 2 and Fig. 3 for the results. Effects of genotype on the percent sucrose consumption on the test day were analyzed with liquid consumption as covariates.

3.3.1. Liquid consumption and body weight as a function of genotype

Genotype differences were observed in liquid consumption on PE1, PE2 and PE3 (respectively, ($F(1,80) = 4.4$, $P = 0.006$), ($F(1,80) = 7.9$, $P = 0.000$) and ($F(1,80) = 7.1$, $P = 0.000$)) (Table 2). On PE1, F2 consumed more than 129 mice ($P = 0.033$). On PE2, F2 consumed more than 129 ($P = 0.000$) and B6

Table 2
Latent inhibition: amount of liquid consumed as a function of genotype and pre-exposure group

Liquid consumption (in ml)	Genotype (NPE/PE)															
	129-NPE		129-PE		B6-NPE		B6-PE		F2-NPE		F2-PE		WT-NPE		WT-PE	
	Y	S.E.M.	*Y*	S.E.M.	*Y*	S.E.M.	*Y*	S.E.M.	*Y*	S.E.M.	*Y*	S.E.M.	*Y*	S.E.M.	*Y*	S.E.M.
PE1	1.02	0.14	1.16	0.14	1.39	0.07	1.64	0.10	1.40	0.14	1.69	0.12	1.07	0.24	1.19	0.26
PE2	1.36	0.24	1.90	0.11	1.70	0.11	1.98	0.12	1.89	0.09	2.51	0.12	2.06	0.10	2.26	0.14
PE3	1.71	0.21	2.11	0.08	1.84	0.15	2.14	0.10	2.06	0.10	2.66	0.12	2.23	0.10	2.51	0.11
CSUCROSE	3.00	0.36	2.28	0.30	2.92	0.33	2.75	0.33	2.35	0.13	2.59	0.12	2.44	0.16	2.76	0.21
TTOTAL	1.75	0.18	1.55	0.22	1.36	0.12	1.82	0.14	1.86	0.19	2.17	0.14	1.73	0.21	1.95	0.11

Pre-exposure days (PE1–3), conditioning day (4, CSUCROSE) and test day (5, TTOTAL); pre-exposed (PE) and non-pre-exposed (NPE) animals; (mean ± S.E.M.)
Y: mean; S.E.M.: standard error of mean.

($P = 0.047$) mice. Also, WT consumed more than 129 mice ($P = 0.001$). On PE3, F2 consumed more than 129 ($P = 0.005$) and B6 ($P = 0.028$) mice. Also, WT consumed more than 129 ($P = 0.003$) and B6 ($P = 0.020$) mice. This could be related to the finding that the genotypes differed in body weight on the conditioning day ($F(1,80) = 7.6$, $P = 0.000$): F2 weighed more than 129 ($P = 0.002$) and B6 mice ($P = 0.000$).

Additionally, on PE2 and PE3, PE mice consumed more than NPE mice (PE2 ($F(1,80) = 18.2$, $P = 0.000$) and PE3 ($F(1,80) = 19.2$, $P = 0.000$)). Effects on consumption did not reach significance on the conditioning and test days. Also, interactions between pre-exposure and genotype did not reach significance.

3.3.2. Sucrose ratio on the test day as a function of genotype

Only the pre-exposure effect reached significance for the sucrose ratio ($F(1,80) = 69.1$, $P = 0.000$), which means that all genotypes showed significant latent inhibition: the sucrose ratio was significantly higher in PE mice in comparison with NPE mice (Fig. 3). The interaction between genotype and pre-exposure was not significant, while covariates failed to attain statistical significance. So, the genotype did not influence latent inhibition.

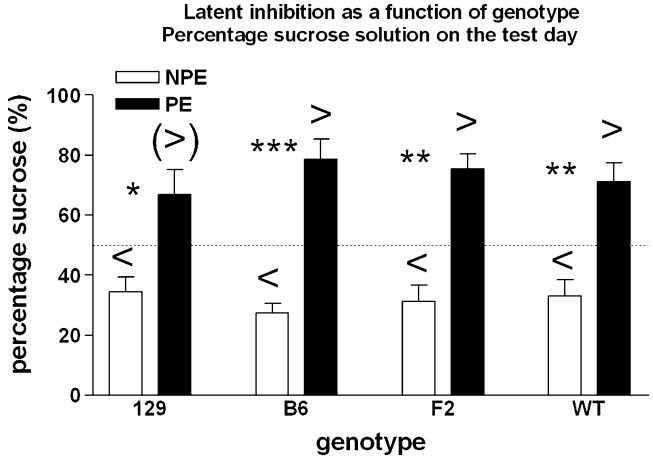

Fig. 3. Sucrose ratio on the test day (mean ± S.E.M.) in the latent inhibition (LI) paradigm in pre-exposed (PE) and non-pre-exposed (NPE) animals as a function of genotype. Difference between PE and NPE group: *** is significant at the 0.001 level (two-tailed); asterisk (**) is significant at the 0.01 level (two-tailed); asterisk (*) is significant at the 0.05 level (two-tailed). *Sucrose ratio*: (<) lower than 50% chance level, conditioned taste aversion (CTA); (=) equal to 50% chance level; (>) higher than 50% chance level, sucrose solution preference.

The mean sucrose ratio was significantly lower than 50% in NPE mice from all genotypes (129 $P = 0.013$; B6 $P = 0.000$, F2 $P = 0.007$, WT $P = 0.012$). So, NPE mice showed conditioned taste aversion. The number of NPE animals with a ratio lower than 50% was 10 in B6, 8 in F2 and WT and 7 in 129 mice. The sucrose ratio was significantly higher than 50% in B6 ($P = 0.002$), F2 ($P = 0.001$) and in the WT ($P = 0.010$) PE mice and tended to be higher than 50% in 129 PE mice ($P = 0.071$). This means that the PE animals showed a preference for the sucrose solution. The number of PE animals with a ratio higher than 50% was 10 in F2, 9 in B6 and WT and 6 in 129 mice.

3.4. Discussion

Genotype differences in behavioral responses were also studied in the latent inhibition paradigm, particularly to establish whether the F2 mice show normal LI in comparison to the other genotypes. All genotypes indeed showed normal latent inhibition using the conditioned taste aversion paradigm. In all genotypes, NPE animals responded with normal CTA for the sucrose solution and PE animals were found to posses a preference for the sucrose solution on the test day.

Genetic differences in lithium – induced conditioned aversion have been examined using taste – conditioning procedures in adult male B6 and DBA/2J mice [58]. In that study, DBA/2J mice showed stronger conditioned taste aversion than B6 mice. Here, we have not observed genotype differences in taste aversion as measured in the NPE mice. WT and particularly the F2 mice consumed more liquid than the other two genotypes on PE2 and PE3. F2 also weighed more than the other genotypes. Additionally, on these days, PE mice (receiving sucrose solution) consumed more than the NPE mice (receiving plain water). The interaction between pre-exposure and genotype did not reach significance, which indicates that genotypes did not differ in sucrose preference, although we did not directly measure sweet preference in a two-bottle preference test. Mouse strains have been found to show large differences in consumption of sweeteners [51]: e.g. studies using two-bottle preference tests have indicated that mice from B6 strains have high avidity and mice from 129 strains have low avidity for sweeteners [1,3,10,43,44].

A number of factors have been suggested to confound drug effects on LI when using CTA instead of other paradigms for measurement of LI and therefore should be taken into account according to Moser et al. [46]. First, genetic manipulation or drugs that reduce or increase fluid consumption will change the amount of pre-exposure. Therefore, we have supplemented statistical analyses with additional analyses of covariance (ANCOVA) to test whether concomitant effects of genotype on drinking behavior as such could account for potential effects on LI. Covariates did not reach significance. Secondly, during pre-exposure, injection of drugs may itself induce CTA. Effects of drugs in a two-bottle preference test could provide an answer to this question. Similarly, drugs may also modify the aversiveness of the LiCl injection. This can only be controlled for by including two additional groups for each experimental condition with animals that are not injected with LiCl. Finally, as indicated by Moser et al. [46], in a CTA protocol, animals are not learning to ignore the sucrose solution during pre-exposure as would be required for LI. In fact quite the opposite: they are learning to appreciate the sweet taste. The use of a neutral CS (taste that mice do not prefer over plain water) is an option to overcome this problem.

Another consideration, depending on the paradigm used to measure LI, different brain regions seem to be involved. For example, results from other studies have shown that the striatum plays a prominent role in the disruption of LI as assessed by CTA. Injections of amphetamine into the striatum, but not the nucleus accumbens, have been shown to impair LI, using the CTA paradigm [24]. Also, it has been found that PE animals had significantly fewer Fos-like immunoreactivity (FLI)-positive cells in the striatum than NPE animals; however, no differences were seen in the nucleus accumbens [66]. In contrast, when using a different paradigm, such as the conditional emotion response (CER) [36], increased dopamine function in the nucleus accumbens has been shown to induce attenuation of LI due to impulse-dependent release of dopamine occurring at the time of conditioning.

In general, it should be taken into account that despite the fact that CTA is easy to use, more studies should be performed in order to pharmacologically validate the test in mice before it can be used for drug screening.

4. Prepulse inhibition experiment

4.1. Introduction

Prepulse inhibition (PPI) refers to the reduction of startle reaction to a startle-eliciting stimulus when it is shortly preceded by a weak stimulus. PPI is also a cross-species phenomenon. PPI may reflect underlying sensorimotor processes involved in the filtering of exteroceptive stimuli for their cognitive or physiological relevance. Deficits in PPI are observed in psychiatric patients, such as schizophrenic patients [6].

Deficits in PPI can also be induced in rodents with administrations of various schizophrenomimetics, such as *d*-amphetamine, apomorphine, phencyclidine (PCP) and ketamine [8,17,18,20,31,53] and disrupted PPI can be normalized by antipsychotic drugs [31]. PPI is used in the development of new CNS drugs. Strain differences in PPI have been reported as well [41,47]. Here, PPI parameters were compared in the four mice genotypes, again particularly to establish whether the F2 mice show normal PPI in comparison to the other genotypes.

4.2. Materials and methods

4.2.1. Animals

Values in the four mouse genotypes were assessed. For this experiment, a new batch of animals were tested. Each experimental group consisted of 10 animals.

4.2.2. Apparatus

The acoustic startle measure is based on the reflexive whole-body flinch, or startle response, following exposure to a sudden noise. Animals were tested with a San Diego Instruments SR-Lab system (San Diego, CA, USA). Mice were placed in a small Plexiglas cylinder within a larger, sound-attenuating chamber. The cylinder was seated upon a piezoelectric transducer, which allowed vibrations to be quantified and displayed on a computer. Response sensitivities were calibrated to be nearly identical in all eight chambers. The chamber included a fan, and a loudspeaker for the acoustic stimuli (bursts of white noise). Background sound levels (70 dB) and calibration of the acoustic stimuli were confirmed with a sound level meter (Rion NL 14).

4.2.3. Procedures

Sessions were structured as follows: (1) 5-min acclimation at background noise level; (2) 10 startle alone trials; (3) actual test session consisting of 72 trials. There were eight different types of trials: the no-stimulus trials, trials with the acoustic startle stimulus alone (a single white noise burst; 40 ms; 120 dB), trials with prepulse stimuli alone (20 ms; either 74, 78 or 82 dB) and trials in which a prepulse stimulus (20 ms; either 74, 78 or 82 dB) had onset 100 ms before the onset of the startle stimulus. The different trial types were presented in 9 blocks of 8, in randomized order within each block, with an average intertrial interval of 15 s (range: 10–20 s). At the onset of the stimulus, 150 1-ms readings were collected, the mean of which defined the startle magnitude. An overall analysis was performed for each subject's data for levels of prepulse inhibition at each prepulse sound level (calculated as $100 - [(\text{response amplitude for prepulse stimulus and startle stimulus together/response amplitude for startle stimulus alone}) \times 100]$). Also, the mean PPI level (irrespective of prepulse intensity) was determined.

4.2.4. Statistical analyses

One-way Anova (SPSS 11.5 Statistical Package) was used to analyze genotype differences in the dependent variables (prepulse inhibition and basal startle response). If appropriate, analyses were followed by post hoc tests according to Tukey to determine differences between genotype groups. Differences were considered as tendencies for $0.05 \leq P \leq 0.1$ and statistically significant for $P < 0.05$.

4.3. Results

See Fig. 4 for the results.

4.3.1. Basal startle and body weight as a function of genotype

Genotypes differed in startle ($F(3,43) = 3.8$, $P = 0.017$) and body weight ($F(3,43) = 13.9$, $P = 0.000$). Particularly, WT mice had a higher basal startle in comparison with the B6 ($P = 0.027$) and F2 ($P = 0.040$) mice. WT weighed more than 129 and B6 mice (both, $P = 0.000$). F2 weighed more than 129 animals ($P = 0.013$).

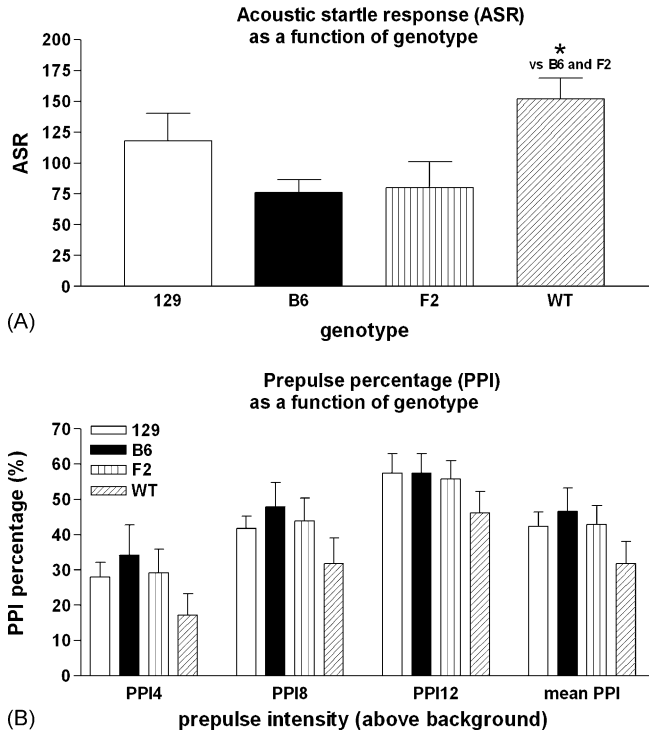

Fig. 4. Prepulse inhibition parameters (mean ± S.E.M.) as a function of genotype: basal acoustic startle response (ASR, A) and prepulse inhibition percentage (PPI, B) in trials with different prepulse intensities (PPI4, PPI8, PPI12) and the mean PPI. * is significant at the 0.05 level (two-tailed).

4.3.2. Prepulse inhibition as a function of genotype

No differences between genotypes were found in PPI percentages (PPI4 ($F(3,43) = 1.2$, $P = 0.325$); PPI8 ($F(3,43) = 1.2$, $P = 0.324$); PPI12 ($F(3,43) = 0.95$, $P = 0.427$); mean PPI ($F(3,43) = 1.3$, $P = 0.302$)).

4.4. Discussion

When comparing the four genotypes, no differences were found in PPI levels. This meant that PPI in the F2 mice did not deviate from PPI levels in the remaining mice. ASR was increased in the WT compared to the B6 and F2 mice.

5. Locomotor activity experiment

5.1. Introduction

Spontaneous locomotion was measured in the four mice genotypes to control for differences in general activity levels.

5.2. Materials and methods

5.2.1. Animals

The four mice genotypes were compared on spontaneous locomotor activity: B6, 129, F2 and WT male mice. For this experiment, a new batch of animals were tested. Each experimental group consisted of 10 animals.

5.2.2. Apparatus and procedures

Locomotion was assessed in two setups with each four non-transparant plastic cylinders (internal diameter of 21 cm and a height of 30 cm). A computerized tracking system and image analyzer (EthoVision® 3.0.15, Noldus, Wageningen, The Netherlands) was used to monitor walking patterns. The camera hung perpendicular to the center of the setup. The image analyzer tracked the center of each mouse with a sampling rate of 25 Hz, and allowed the calculation of distance traveled (in cm). Locomotion was monitored for 60 min for each animal.

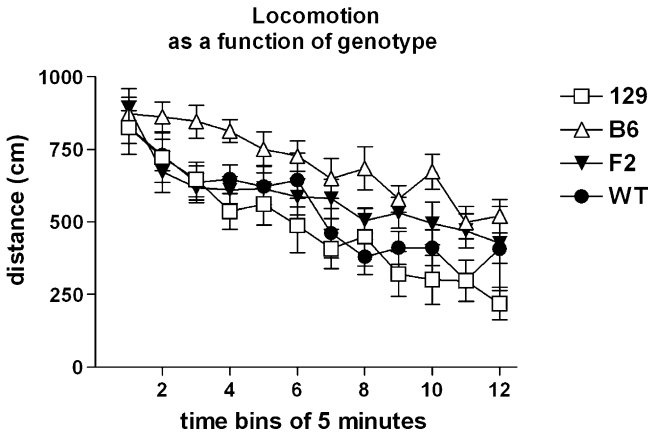

Fig. 5. Locomotor activity (LMA) as a function of genotype: distance traveled in activity boxes (mean ± S.E.M., in cm, 5 min time bins).

5.2.3. Statistical analyses

MANOVA General Linear Model (GLM)-analysis (SPSS 11.5 Statistical Package) was used to determine the effects of the between subject factor (genotype), the within subject factor (block of 20 min) and dose by block interaction on the dependent variable (distance traveled). One-way ANOVA was used to analyze group differences on total distance traveled and distance per block of 20 min. If appropriate, analyses were followed by post hoc tests according to Tukey to determine differences between genotype groups. Differences were considered as tendencies for $0.05 \leq P \leq 0.1$ and statistically significant for $P < 0.05$.

5.3. Results

See Fig. 5 for the results.

Locomotor activity significantly decreased over time blocks of 20 min ($F(2,72) = 90.0$, $P = 0.000$). Genotype differences were found in locomotor activity ($F(3,36) = 3.2$, $P = 0.036$): total locomotion was significantly higher in B6 in comparison with 129 mice ($P = 0.026$). The interactions between time block and genotype did not reach significance.

5.4. Discussion

Finally, there was a genotype difference in total locomotion. In particular, B6 mice were hyperactive in comparison with 129 mice, which is in line with previously reported data [45]. They did not differ from the other two genotypes.

6. General conclusions

Table 3 gives an overview/summary of the effects of genotype on Morris water maze performance, latent inhibition, prepulse inhibition and locomotor activity.

The Morris water maze as measured in the present study can potentially be considered as a preclinical test to measure visual spatial learning and memory capabilities in mice. Here,

Table 3
Summary results: different test parameters as a function of genotype

Morris water maze acquisition	
Latency	F2 < 129, B6, WT
Distance	F2 < 129, WT
Periphery	F2 < 129, B6, WT
Velocity	–
Morris water maze probe trial	
Target	129 < F2 129 at chance level
Crossings	–
Velocity	B6 < F2
Start	F2 < 129
Latent inhibition	
NPE/PE Difference	Normal: F2 = 129 = B6 = WT
NPE/CTA	Normal CTA: F2 = 129 = B6 = WT
PE/Sucrose pref.	Normal sucrose preference: F2 = 129 = B6 = WT
Liquid cons.	129 (PE1–3), B6 (PE2–3) < F2 129 (PE2–3), B6 (PE3) < WT
Prepulse inhibition	
PPI	Normal: F2 = 129 = B6 = WT
Startle	F2, B6 < WT
Locomotion	
Spont. Locom.	129 < B6

we have shown that the F2 generation of 129xB6 crosses provides a control sample with good baseline performance. Results were verified by comparison against a large in house database of mice tested under identical conditions irrespective of different solvent treatment-schedules. Furthermore, it was shown that reliable deficits could be obtained in learning and memory in this paradigm following injections with scopolamine (0.16 mg/kg) in a larger sample of F2 animals. Therefore, this deficit model in F2 mice can be used to test effects of reference and novel compounds in order to develop cognitive enhancing drugs. Besides the Morris water maze, tests measuring other aspects of cognition are under development, such as the five choice serial reaction time task (5-CSRTT, measure of attention), the object recognition test (measuring episodic [working] memory) and social recognition test (test for social cognition). For example, we have tested the F2 mice in the 5-CSRTT and we have found that these mice perform excellent in this task as well (unpublished data, in prep.).

LI has been suggested to predict antipsychotic activity, "typical" versus "atypical" action of antipsychotic drugs (APDs) and effectiveness against negative symptoms under different test conditions [69], according to Weiner. They describe this as the "two-headed LI model, which mimics two extremes of deficient cognitive switching seen in schizophrenia". This has been referred to as "excessive and retarded switching between associations, that can serve to model positive symptoms of schizophrenia and typical antipsychotic action, as well as negative symptoms of schizophrenia and atypical antipsychotic action". Lubow directly relates LI to the operation of selective attentional processes that are dysfunctional in schizophrenia [42], while others favor a framework in which disruption of LI and blocking in acute schizophrenics is viewed as an inability to compare and express stored representations (i.e. associative performance deficit) [25]. In the present study, we have shown that all mice genotypes showed normal latent inhibition using the conditioned taste aversion paradigm. It will be interesting to further study and characterize the effects of drugs in mice in the CTA paradigm in order to establish the construct validity of the LI model.

The paradigm that has been most often used to investigate pre-attentive processing and has been found to be disrupted in schizophrenia, prepulse inhibition, has been suggested to have the advantage that it can be measured in humans and animals with virtually identical methods [7,23]. Here, no mice genotype differences were found in PPI levels. However, different susceptibility to PPI-disruptive drugs and reversal remains to be determined.

Following the setup of these procedures in mice, we are now able to compare the effects of gene knockout/mutations used for target validation with results in the present study as a frame of reference.

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