Young, Matthew J. and Geiszler, Philippine C. and Pardon, Marie-Christine (2016) A novel role for the immunophilin FKBP52 in motor coordination. Behavioural Brain Research, 313 . pp. 97-110. ISSN 1872-7549

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PII: S0166-4328(16)30443-0
DOI: http://dx.doi.org/doi:10.1016/j.bbr.2016.07.015
Reference: BBR 10322

To appear in: *Behavioural Brain Research*

Received date: 28-5-2016
Revised date: 9-7-2016
Accepted date: 10-7-2016

Please cite this article as: Young Matthew J, Geiszler Philippine C, Pardon Marie-Christine.A novel role for the immunophilin FKBP52 in motor coordination.*Behavioural Brain Research* http://dx.doi.org/10.1016/j.bbr.2016.07.015

A novel role for the immunophilin FKBP52 in motor coordination

Running title: FKBP52 and cognition

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Highlights

- The immunophilin FKBP52 is known to modulate hallmarks of Alzheimer's disease
- Downregulation of FKBP52 levels does not alter cognitive performance with ageing
- Reduced FKBP52 levels lead to impaired motor coordination

Abstract

FKBP52 is a ubiquitously distributed immunophilin that has been associated with wide-ranging functions in cell signalling as well as hormonal and stress responses. Amongst other pathways, it acts *via* complex-formation with corticosteroid receptors and has consequently been associated with stress- and age- related **neurodegenerative disorders including Alzheimer's and Parkinson's diseases.** Reduced levels of FKBP52 have been linked to **tau dysfunction** and amyloid beta toxicity in AD. However, FKBP52's role in cognition and **neurodegenerative disorder**-like phenotypes remained to be elucidated.

The present study aimed therefore at investigating the cognitive and behavioural effects of reduced FKBP52 levels of genetically modified mice during ageing. Female and male $FKBP52^{+/+}$, $FKBP52^{+/-}$ and $FKBP52^{-/-}$ mice were compared at two-, ten-, twelve-, fifteen- and eighteen-months-of-age in a series of behavioural tests covering specie-specific behaviour, motor activity and coordination, fear-, spatial and recognition memory as well as curiosity and emotionality.

Whilst cognitively unimpaired, $FKBP52^{+/-}$ mice performed worse on an accelerating rotating rod than $FKBP52^{+/+}$ littermates across all age-groups suggesting that FKBP52 is involved in processes controlling motor coordination. This deficit did not exacerbate with age but did worsen with repeated testing; pointing towards a role for FKBP52 in learning of tasks requiring motor coordination abilities.

This study contributes to the knowledge base of FKBP52's implication in neurodegenerative diseases by demonstrating that FKBP52 by itself does not directly affect cognition and may therefore rather play an indirect, modulatory role in the functional pathology of AD, **whereas it directly affects motor coordination, an early sign of neurodegenerative damages to the brain.**

keywords: FKBP52, behaviour, cognition, rotarod, motor coordination

1 Introduction

FKBP52 is classed as an FK506-binding immunophilin of high molecular mass (52 kDa) [1] expressed in a variety of mammalian tissue cells including nervous cells [2]. With its tetratricopeptide repeat domains in its C-terminus part, it acts as a chaperone in protein folding; and partakes in the hormonal metabolism by binding to progesterone- and androgen-receptors [1, 3]. Its peptidyl-propyl *cis/trans* isomerase domain is necessary for its interaction with glucocorticoid receptors (GRs) [1, 4]. **FKBP52 participates to the nuclear transport of and activation of GRs [5],** and as such, is involved in the stress system [6, 7]. **FKBP52 also binds to the mineralocorticoid receptor-heat shock protein 90 (MR-Hsp90) complex [8] favouring the cytoplasmic transport of MR to the nucleus [9]. By regulating MR function, FKBP52 can thus modulate neuronal function and survival as well as behaviours related to stress, mood and learning [10].**

As a glucocorticoid receptor **regulator**, FKBP52 is implicated in the hypothalamic-pituitary-adrenal axis [4] which is crucial for brain ageing; and higher levels of FKBP52 have been measured in aged (twenty-six-month-old) C57Bl/6N mice when compared to six-month-old C57Bl/6N mice [11]. This age-related FKBP52 increase is not associated with recognition memory performance in senescent mice [11]. FKBP52 is expressed in brain areas such as in the hippocampus, frontal cortex, amygdala and basal ganglia [12] **which are also implicated in the pathology of a number of neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease**. Traditionally, AD is characterised by accumulation and aggregation of two proteins, namely amyloid beta (Aβ) and tau. Small soluble Aβ aggregates and neurofibrillary tangles (created by tau aggregation) appear to be good indicators of AD severity [13]. FKBP52 has shown to interact with both proteins, whereby it modulates Aβ toxicity [12] and prevents microtubule formation by tau [14]. FKBP52 expression has been found to be particularly low in the frontal cortex of deceased AD patients [15]. This suggests that reduced FKBP52 levels are a risk factor for cognitive decline.

Whilst FKBP52 has been found to alter Aβ or tau processing in cell cultures [12, 14] but it is it is unclear to what extent this affects functional aspects that are traditionally associated with Aβ or tau accumulations, *e.g.* cognitive decline. Furthermore, FKBP52

levels are altered in the ageing brain [11], and therefore this may affect the severity of behavioural decline in ageing and/or behaviours relevant to neurodegenerative diseases other than AD. The aim of the present study was, therefore, to determine cognitive and behavioural effects of reduced FKBP52 levels in behavioural ageing *per se* using a comprehensive test battery covering species-specific behaviour, motor coordination, locomotor activity, spatial and recognition memory as well as fear learning and memory, sensory motor gating and emotionality. Given the association of FKBP52 with the reproductive system [3] female and male FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice were compared.

2 Materials and methods

2.1 Animals

FKBP52$^{+/-}$ mice, generated as previously described [16], were obtained from the Indiana University School of Medicine (Indianapolis, USA). FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice were bred on a mixed 129SvJ x C57Bl/6J background at the Bio Support Unit of the University of Nottingham (Nottingham, UK). Genotyping was carried out by Transnetyx (Cordova, USA). Separated by sex, animals were group-housed in individually ventilated cages (38 cm x 20 cm x 13 cm). These were supplied with *ad libitum* food and water, sawdust flooring, bedding material, cardboard tubes and wooden chewing sticks for environmental enrichment. Temperature, relative humidity and air exchange were kept consistent in holding rooms and behavioural testing suites. Lighting in holding rooms was maintained at a 12/12, 24 hour light/dark cycle, with lights on at 07h00. All procedures were performed according to the Animals (Scientific Procedures) Act 1986, under project license 40/3601; and reported according to the ARRIVE guidelines [17].

The number of mice used for all three experiments are listed in table 1. Power analysis based on preliminary accelerod data acquired from ten-month-old female and male FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice suggested the use of twelve mice per genotype and age-group in order to detect a genotype-related difference with a 90 % power. Throughout the study, 13 fifteen-month-old and twelve eighteen-month-old mice were lost due to spontaneous death. In experiment 3, six female FKBP52$^{-/-}$ mice were compared to six female FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice. The five male FKBP52$^{-/-}$ mice, however, displayed inter-sex characteristics

externally, with underdeveloped testicles upon dissection, and their ambiguous sex questioned the use of males as appropriate controls

2.2 Experimental design

The initial experiment tested ten- and twelve-month-old mice of both sexes towards the end of their reproductive period in a longitudinal study (experiment 1). Genotype differences that evolved between these two age groups could either be ascribed to advancing age or repeated testing. Hence, in experiment 2, experimentally naïve male and female FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice underwent the same tests at two-, twelve-, fifteen- and eighteen- months-of-age, covering the beginning and end of the reproductive period, as well as senescence, to confirm whether these effects were persistent and exacerbated with advanced age. The ideal of observing FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice over a period of time to study the consequences of FKBP52 reduction and loss could not be realised due to breeding issues. It appeared that the majority of FKBP52$^{-/-}$ mice died at birth, to the effect that only 12 FKBP52$^{-/-}$ mice out of a 1300-mouse-strong colony survived birth; confirming previously reported breeding problems [18]. The surviving homozygous knock-outs were tested together with FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice at twelve-months-of-age only (experiment 3) and were used to confirm the results that were obtained from experiment 2. Figure 1 depicts the order of tests that were applied in order of increasing severity [19].

2.3 Statistical analysis

Data are presented as mean ± SEM. All analyses of variance and covariance (ANOVA and ANCOVA, respectively), as well as mixed model repeated measures analyses in the following were conducted using InVivoStat (v. 2.5; Geiszler, Barron [20]). One-sample *t*-tests were performed using SPSS v. 21.0.0.0 (IBM SPSS Statistics). An effect was considered significant when p values were ≤ 0.05 and *post-hoc* planned comparisons were used where appropriate. Only statistically significant effects due to genotype or its interactions with other factors (e.g. age or sex) are illustrated in the main manuscript. Remaining data is presented in the supplementary material. Data collected in experiment 3 was graphically represented and described but not statistically compared due to the sexual ambiguity of FKBP52$^{-/-}$ male mice.

2.4 Body mass

All animals were weighed before they went on experiment on day 0, and again on days 4 and 14. In experiment 1, day 0 data were analysed by three-way mixed model with repeated measures; with genotype and sex as between-subject factors and age as the repeated factor. In experiment 2, day 0 data were analysed by single-measures three-way ANOVA, with genotype, age and sex as between-subject factors.

2.5 Behavioural and cognitive tests

2.5.1 Food burrowing

Food burrowing assesses a species-specific behavioural analogue of daily living activity [21, 22]. For the duration of this test, mice were singly housed in individually ventilated cages that were equipped like their home cages. In addition to their usual diet, mice were presented with a glass jar that contained small pellet, high-nutrient food (50 g). The percentage of food displaced from each jar between 5 pm and 9 am the following morning was recorded. Food displacement data were analysed by a three-way mixed model approach with repeated measures in experiment 1; using genotype, age and sex as between-subject factors and body mass as covariate. Data from experiment 2 were analysed by a three-way ANCOVA, with genotype, age and sex as between-subject factors and body mass as covariate.

2.5.2 Spontaneous alternation

The spontaneous alternation performance was tested in a Y-shaped maze. In this study the maze was made of transparent polyacrylate (44 cm long x 7 cm wide x 25 cm high; three identical arms 120° apart). Mice were placed in the centre and allowed to move freely for 5 minutes. The number of arms entered was counted as a measures of locomotor activity and exploratory behaviour [23]. Entries into an arm that differed from the previous two were accepted as successful alternation, indexing spatial short-term working memory [24, 25]. The alternation rate (%) was calculated as follows: 100 x number of alternations / (number of arm entries – 1). The number of arms entered and alternation rate were analysed by either three-way mixed model repeated measures approach (experiment 1); with genotype and sex as between subject-factors and age as the repeated factor; or by three-way single measures ANOVA (experiment 2), with genotype, age and sex as between-subject factors. An

alternation rate significantly above 50 % (random chance) indicates the use of a spatial working memory strategy [26] which was tested using a one sample t-test in all three experiments.

2.5.3 Open field and novel object

Mice were first individually habituated to the empty open-field arena (35 cm x 30 cm x30 cm height) for 30 minutes. Ethovision tracking software (v. 7.0; Noldus, Wageningen, Netherlands) was used to acquire the distance mice moved as measurement of locomotor activity [27]. To evaluate anxiety-like behaviour [27], the relative distance they moved in the centre of the arena (21 cm x 6 cm) was calculated [28] to ensure that any differences in object exploration levels between treatment groups were due to differences in memory and not activity and/or anxiety levels.

The novel object tasks were used to assess location and recognition memory on day 2. These tasks exploit the natural tendency of mice to preferentially explore novel, over familiar objects [29] and their locations. The procedure used here was adapted from a previously validated protocol [30]. The objects were striped, wooden, prisms with a circular or triangular area. All mice were subjected to 3 trials over a period of 36 minutes. In the habituation trial, mice were presented with two identical objects placed adjacent to each other for six minutes. Ten minutes later, the mice were presented with the same objects – albeit one had moved to the opposite corner in the arena – for another six minutes (location trial). Twenty minutes after the second trial, the mice were returned to the arena for the discrimination trial where one of the two objects was replaced by an object of a different shape (discrimination trial). Ethovision tracking software was used to record the duration of exploration of each object in each trial, where exploration was defined as detection of the nose-point $\leq$ 1 cm from each object. Mice exhibiting a total exploration time of less than five seconds in any one trial were excluded from analysis due to insufficient exploration.

Open field and novel object data were analysed by either a three-way mixed model repeated measures approach (experiment 1); with genotype and sex as between-subject factors and age as the repeated factor; or by a three-way single measure ANOVA (experiment 2) with genotype, age and sex as between-subject factors. The preference indices were also compared to chance levels (50 %) using a one sample t-test.

2.5.4 Accelerod

Motor coordination was assessed using a modified rotarod test protocol [31-33]. The accelerating rotarod (Harvard apparatus) consisted of a rotating, white, ridged rubber rod, separated by fixed, white polyacrylate partitions into five, 6-cm-wide sections. Accelerod experiments were carried out in three trials, consisting of two runs each, over three days, in which each mouse was placed onto the rod which rotated at a baseline speed of 4 rpm. Over the following ten minutes time the rod steadily accelerated up to a maximum of 40 rpm. The latency to fall from the accelerating rod indicated motor coordination performance. This was noted for each run and subsequently averaged for each trial. If mice reached the maximum limit of ten minutes, they were removed from the rod and given the maximum time. The effect of age or repeated testing on latency to fall in experiment 1 was assessed by calculation of a performance index, where performance index = (trial 9 latency – trial 1 latency)/trial 9 latency x 100.

Latency to fall acquired in experiment 1 was analysed within each age group by three-way mixed model with repeated measures; with genotype and sex as between-subject factors and trial as the repeated factor. The performance index was analysed by three-way mixed model repeated measures approach using genotype and sex as in-between factors and age as repeated factor. Latency to fall data acquired in experiment 2 were analysed by four-way mixed model with repeated measures; using genotype, age and sex as between-subject factors and trial as the repeated factor. Body mass was considered as covariate in all analyses.

2.5.5 Acoustic startle response and prepulse inhibition

The startle response comprises a contraction of skeletal muscles elicited by a sudden, intense stimulus [34]. If this stimulus is preceded by a non-startling stimulus the magnitude of the response can be reduced (prepulse inhibition); which is understood to measure sensorimotor gating and the ability to process information [34]. The test protocol used here was adapted from [35]. Acoustic stimuli were delivered and startle responses measured via a piezoelectric sensor in a two-unit automated startle system (SR Lab software; San Diego Instruments, San Diego, CA, USA). The startling pulse consisted of a single white noise burst (40 ms at 120 dB). The prepulse + pulse trials consisted of a prepulse of noise (20 ms at 68, 72, 80 and 90 dB respectively) followed by startling pulse 100 ms after prepulse onset. The protocol began with a five-minute-long acclimation period at background noise level (no-stimulus). The following 84 trials consisted of 24 startling pulse trials, 12 no-stimulus trials and 48 prepulse + pulse trials (12 of each prepulse amplitude) in pseudorandom order. Inter-trial intervals

were pseudo-randomly distributed between 12-30 seconds. The indices measured from raw output data were startle latency, startle magnitude and % prepulse inhibition [100*(prepulse + pulse amplitude/pulse amplitude alone)] to assess the mice's responsiveness to stressful situations [36].

Startle latency and startle magnitude were analysed by either a three-way mixed model approach with repeated measures; with genotype and sex as between-subject factors and age as the repeated factor (experiment 1) or by three-way single measure ANCOVA, with genotype, age and sex as between-subject factors (experiment 2). Body mass was entered as covariate in all cases. % prepulse inhibition data of experiment 1 were analysed within each age group by three-way mixed model approach with repeated measures; with genotype and sex as between-subject factors and dB level of the prepulse as repeated factor, whereas data of experiment 2 were analysed by four-way mixed model repeated measures approach; with genotype, age and sex as between-subject factors and prepulse dB level as repeated. Body mass was used as covariate.

2.5.6 Contextual fear conditioning

The protocol used here has been described previously [28] and was used to assess acquisition, retention and extinction of contextual memory [37].

On each day of this three-day-long experiment, mice were placed individually in a test chamber (25 cm x 22 cm) consisting of a metal grid floor, three metal walls and one clear polyacrylic wall. The Ethovision tracking software recorded the time mice spent immobile, which was defined as less than 0.75% change of mouse "area" as viewed from above. For the acquisition trial, mice were placed into the test chamber and administered electric foot shocks every minute (0.4 mA, one-second-long, every minute) for ten minutes. An increase in the time the mice spent immobile with incrementing number of shocks indicated positive learning of the averseness of the context (the chamber).

In experiment 1, the effect of genotype and sex on associative learning was assessed per age-group. The statistical analysis included three-way mixed model approach with repeated measures; with genotype and sex as between-subject factors and number of shocks as the repeated factor. In experiment 2, a four-way mixed model repeated measures strategy analysed immobility data; with genotype, age and sex as between-subject factors and number of shocks as repeated factor.

Retention and extinction trials were carried out after a 24- and 48-hour-long delay, respectively, whereby mice were presented with the same test chamber for three-minutes without receiving foot shocks. The length of time the mice spent immobile in the second trial was used as a measure of retention of contextual fear memory. The extinction index (time immobile during extinction trial minus immobility time during retention trial) indicated extinction of contextual fear memory. A negative value suggested successful extinction.

Immobility data of the retention and extinction trials as well as the extinction indices were analysed by a three-way repeated measures mixed model strategy; with genotype and sex as between-subject factors and age as repeated factor in experiment 1; whilst data obtained from experiment 2 were analysed by three-way single measure ANOVA (genotype, age and sex as between-subject factors). The difference of extinction index values from 0 was assessed by student's t-test in all experiments.

2.5.7 Elevated plus maze

The elevated plus maze test was used to assess anxiety-like behaviour in mice by exploiting their natural aversion for open, elevated spaces and their conflicting desire to explore novel surroundings [38]. This test was performed according to a previously described procedure [7]. The plus-maze was made of white polyacrylate, consisting of four arms (5 cm x 35 cm each); two 'closed' arms with white walls (12 cm high) and two 'open' arms without any walls; held at a one-meter-high elevation. Each mouse was left to explore the maze for five minutes which was tracked by Ethovision. The percentage of the time the mice spent in the open arms over closed arms was calculated and used as a measure of anxiety-like behaviour. Mice that fell from the maze during their trial were excluded from the analysis.

The percentage of time that the mice spent in the open arm was analysed by a three-way repeated measures mixed model strategy with genotype and sex as between-subject factors and age as repeated factor in experiment 1. Data obtained from experiment 2 were analysed by three-way single measure ANOVA, where genotype, age and sex were used as between-subject factors.

3 Results

The focus of the study was the impact of FKBP52 downregulation with ageing. All AN(C)OVA and mixed model repeated measures analysis results are listed in the supplementary material (Supplementary Tables 1 and 2). Table 2 summarizes the significant effects of genotype in the test battery, which are described in detail below. Age- and sex-related effects, independent of genotype, are detailed in the supplementary material.

3.1 Body mass

In the first experiment, the mice's body mass increased between ten- and twelve-months-of-age irrespective of sex (age: $F_{(1, 43)} = 101.67$, $p < 0.0001$; Figure 2A). The effect of genotype on the mice's body mass ($F_{(1, 43)} = 5.28$, $p = 0.0265$) was solely due to females. Female FKBP52$^{+/-}$ mice were heavier than their FKBP52$^{+/+}$ littermates ($p = 0.0084$ and $p = 0.0043$ at ten and twelve-months-of-age, respectively), although the genotype x sex interaction failed to reach the critical level of significance ($F_{(1, 43)} = 3.3$, $p = 0.0761$).

The age-dependent increase in body mass was confirmed when considering a wider age range, from two- to eighteen-months-of-age (experiment 2; age: $F_{(3, 151)} = 136.05$, $p < 0.0001$); with twelve-, fifteen- and eighteen-month-old mice being heavier than two-month-old mice ($p < 0.001$ in all cases); and eighteen-month-old male mice also being heavier than twelve- and fifteen-month-old male mice ($p = 0.0001$ and $p < 0.0001$, respectively; Figure 2B). The genotype-related difference in body mass seen in experiment 1, was, however, no longer apparent across the wider age range in experiment 2 or at twelve-months-of-age in experiment 3 (Figure 2C).

Overall, downregulation of FKBP52 levels did not affect body mass regardless of age.

3.2 Food burrowing

Food burrowing performance was not affected by genotype in any of the three experiments (Supplementary Figures S1 A, B and C); but showed a trend towards a decline from two- to eighteen-months-of-age (experiment 2: $F_{(3, 150)} = 2.53$, $p = 0.0594$; S1 B). The decrease of food displaced with advancing age is consistent with previous observations (*e.g.* [20]), but performance may be affected by the age-related increase in body mass; as this variable was found to be significant covariate in the statistical analysis ($p = 0.0238$).

3.3 Spontaneous alternation

In experiment 1, ten- and twelve-month-old FKBP52$^{+/-}$ mice visited as many arms of the Y-maze as age-matched FKBP52$^{+/+}$ mice (Figure 3A). By contrast, statistical analysis of two-, twelve-, fifteen- and eighteen-month-old naïve FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice of experiment 2 indicated that the number of arms entered was dependent on their genotype and age ($F_{(3,\ 151)} = 2.99$, $p = 0.033$). This was only significant at the fifteen-months-of-age level where female FKBP52$^{+/-}$ mice visited more arms than their FKBP52$^{+/+}$ littermates ($p = 0.0062$; Figure 3B).

Results of experiment 1 suggested that the number of arms visited was affected by mouse age and sex irrespective of genotype (age x sex: $F_{(1,\ 43)} = 13.92$, $p = 0.0006$, Figure 3A). This result was due to ten-month-old male mice visiting less arms than their female counterparts ($p = 0.0288$) and twelve-month-old male mice ($p = 0.0005$); the latter of which visited more than age-matched females ($p = 0.0153$). In experiment 2, the number of arms entered decreased with age ($F_{(3,\ 151)} = 15.7$, $p < 0.0001$) and was affected by sex ($F_{(1,151)} = 4.96$, $p = 0.0275$) but not by their interaction. At age-level, sex differences were only noted in FKBP52$^{+/+}$ mice at fifteen-months-of-age ($p = 0.0292$; Figure 3B).

In summary, downregulation of FKBP52 expression had no consistent effect on the mice's willingness to explore the Y maze or their spatial working memory. In experiments 1, 2 and 3, all mice alternated arm entries equally and in most cases above chance levels (Supplementary Figures S2 A, B and C, respectively).

3.4 Open field

FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice displayed a similar levels of ambulation in the open field in experiment 1, 2, and 3. The distance travelled in the arena declined from two- to eighteen-months-of-age (experiment 2; $F_{(3,\ 151)} = 13.78$, $p < 0.0001$; Figure 4). Twelve-month-old female FKBP52$^{-/-}$ mice of experiment 3, however, covered a greater distance than their female FKBP52$^{+/+}$ and FKBP52$^{+/-}$ littermates which was comparable to the performance of male FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice. Conversely, twelve-month-old male FKBP52$^{-/-}$ mice (presenting characteristics of prostate dysgenesis) appeared to be less active than male FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice but the opposite was observed in female. In summary, partial knock-down of FKBP52 did not appear to affect locomotor activity. Anxiety-related

behaviour, assessed through the relative distance covered in the centre of the open field, was not affected by the mice's genotype (Supplementary Figures S3 A, B and C).

3.5 Novel object location and recognition

The total time the mice spent exploring both objects was not influenced by the mice's genotype in any of the three experiments, regardless of the trial (Supplementary Figures S4 A - I). During the habituation trial, mice equally explored both objects, regardless of their location, in all three experiments (Supplementary Tables T1 and T2). There was no effect of the genotype on discrimination of the novel location (Supplementary Figures S5 A, B and C).

With regards of object recognition, in experiment 1, where mice were repeatedly tested at ten- and twelve-months-of-age, heterozygosity for FKBP52 affected the preference for the novel over familiar object ($F_{(1,43)}$ = 4.34, p = 0.0435); whereby twelve-month-old female FKBP52$^{+/-}$ mice explored the novel object less than their FKBP52$^{+/+}$ littermates (p = 0.0435; Figure 5A). This difference was not significant in females at ten-months-of-age or in male mice. Experimentally naïve mice of experiment 2 or 3 (Figures 5 B and C) were generally unable to discriminate the novel over familiar object, regardless of their age.

In summary, the data collected here does not support a role for FKBP52 in object exploration or discrimination and spatial discrimination.

3.6 Accelerod

The time the mice spent on the accelerating rod increased with rising number of trials the mice were subjected to; suggesting that all groups improved their motor coordination with training in experiment 1 at ten- ($F_{(8, 272)}$ = 38.6, p < 0.0010; Figures 6A and E) and twelve-months-of-age ($F_{(8, 272)}$ = 14.56, p < 0.0010; Figures 6B and F) as well as in experiment 2 ($F_{(8, 1200)}$ = 69.68, p < 0.0010 Figures 7A - H) and experiment 3 (visual inspection Figures 6D and H). In all experiments this improvement was affected by the mice's genotype in an age-dependent manner: experiment 1 (performance index: genotype x age: $F_{(1, 33)}$ = 4.25, p = 0.0472) and experiment 2 (genotype x age x trial: $F_{(24, 1200)}$ = 2.26, p = 0.0005); and was also influenced by the mice's sex in experiment 1 (genotype x sex x trial: $F_{(8, 272)}$ = 2.02, p = 0.0441) and 2 (age x sex x trial: $F_{(24, 1200)}$ = 1.82, p = 0.0089).

The genotype-related differences became apparent in ten- and twelve-month-old female mice of experiment 1, whereby FKBP52$^{+/-}$ mice fell from the accelerating rod before FKBP52$^{+/+}$ mice in the final trial (p = 0.0333, and p = 0.0394, respectively, Figures 6A and 6B). Similarly, twelve-month-old male FKBP52$^{+/-}$ mice fell from the rod before their FKBP52$^{+/+}$ littermates in trial 5, 6, 7, 8 and 9 (p = 0.0305, p = 0.0297, p = 0.0192, p = 0.0151, p = 0.0154 and p = 0.0157, respectively, Figures 6F). The performance index showed that twelve-month-old female FKBP52$^{+/-}$ mice performed worse than ten-month-old female FKBP52$^{+/-}$ mice (p = 0.0014), as did their male counterparts (p = 0.0557; Figures 6C and G).

Considering a wider age range in experiment 2, experimentally naïve fifteen-month-old mice stayed on the rod for a shorter time than two-month-old mice (p = 0.043, Figures 7C and 7G). Within each age-group, female FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice stayed on the accelerating rod for a similar length of time; except at two-months-of-age, where FKBP52$^{+/-}$ mice remained on it for longer than FKBP52$^{+/+}$ mice in the very first trial (p = 0.0414, Figure 7A). A different picture emerged in male mice where two-month-old male FKBP52$^{+/-}$ mice performed overall worse than their FKBP52$^{+/+}$ littermates which was significant in trial 5 and 7 (p = 0.0139 and p = 0.285, respectively, Figure 7E). Conversely, twelve-month-old FKBP52$^{+/-}$ mice stayed on the rod for longer than FKBP52$^{+/+}$ mice in trial 5 (p = 0.0424, Figure 7F). At eighteen-months-of-age FKBP52$^{+/-}$ mice fell off the rod before FKBP52$^{+/+}$ mice in trial 9 (p = 0.0214; Figures 7H).

In experiment 3, twelve-month-old female FKBP52$^{-/-}$ mice stayed on the accelerating rod for longer than FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice (visual inspection). The latter two groups appeared to perform similarly in both sexes (Figures 6 D and H).

Overall, knock-down of FKBP52 expression affected the mice's motor coordination negatively by reducing the duration the mice stayed on the accelerating rotating rod. This has been seen in both sexes and age-groups investigated. The effect was unrelated to a change in motor activity as observed in the open field; and it was stronger when mice were repeatedly tested at ten- and twelve-months-of age.

3.7 Acoustic startle response and prepulse inhibition

In experiment 1, the latency to startle was statistically affected by genotype as function of age and sex ($F_{(1, 42)}$ = 6.47, p = 0.0147, Figure 8A). A genotype-effect was not observed in experimentally naïve mice ranging from two- to eighteen-months-of-age (experiment 2; Figure 8B) or in experiment 3 comprising twelve-month-old FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice (Figure 8C). The magnitude of the startle response as well as prepulse inhibition was not altered by the mice's genotype in experiments 1, 2, and 3; but decreased from two- to eighteen-months-of-age in experiment 2 (age: $F_{(3,150)}$ = 6.32, p = 0.0005; Supplementary Figures **S6 and S7**).

In summary, the acoustic startle response and its inhibition were not affected by knocking down FKBP52 in mice.

3.8 Contextual fear conditioning

Immobility times and extinction index obtained from the three contextual fear conditioning experiments were not affected by the mice's genotype (see Supplementary Figures S8, S9, S10 and S11; suggesting no role for FKBP52 in fear acquisition, memory and extinction.

3.9 Elevated plus maze

ANOVA results of experiment 2 indicated that genotype affected the time the mice spent on the open arm of the elevated plus maze in an age-dependent manner ($F_{(3, 147)}$ = 4.08, p = 0.0081), but this was only significant at the eighteen-month-old group level where FKBP52$^{+/+}$ males showed higher preference for open arms, compared to FKBP52$^{+/-}$littermates (Figure 9B). Further, within each age group, all mice spent a similar amount of time on the open arm in experiment 1 and 3 (Figures 9A and C, respectively). Thus, knocking down FKBP52 did not result in consistently altered emotionality-related behaviour on the elevated plus maze in mice.

Taken all results of this study together, knocking down FKBP52 in mice worsened performance on an accelerating rotating rod but failed to alter the cognitive parameters tested.

4 Discussion

The present study aimed to assess the behavioural and cognitive phenotype of ageing FKBP52$^{+/-}$ mice; in light of raised FKBP52 levels in senescent C57Bl/6N brains [11] and the potential involvement of FKBP52 in the pathology of age-related diseases with dementia, such as AD [3, 12, 14, 15]. The main question in this regard was whether reduced levels of this protein alone – as found in *post-mortem* AD brains [15] – affected cognition and behaviour of genetically modified mice. Given FKBP52's significance in the reproductive system [3, 39] male and female mice were tested at different age groups covering pre- and post-reproductive periods including senescence.

Our main finding is that FKBP52 deficient mice were cognitively unimpaired and were statistically significantly different from their FKBP52$^{+/+}$ littermates in only a few of the 19 parameters that tested for body mass, species-specific behaviour, locomotor activity, motor coordination and, fear-, object-related and spatial memory as well as curiosity and emotionality. Most consistently, FKBP52$^{+/-}$ mice showed impairments in motor function as tested by an accelerating rotating rod. The behavioural phenotype of FKBP52 null mice was, however, very mild, and given the low number of surviving mice, this suggests that compensatory mechanisms may have taken place in this subset of mice.

The finding of FKBP52$^{+/-}$ mice spending less time on the accelerod than their FKBP52$^{+/+}$ littermates was consistent across the three experiments of the present study. This observation was least prominent in twelve-month-old mice of experiment 3 which might have been due to its reduced statistical power. Experiment 1 demonstrated that repeated testing after two months exacerbated the deficit seen in FKBP52$^{+/-}$ mice. Hence, FKBP52$^{+/-}$ mice's untimely falls from the rod were likely due to a compromised ability to learn within a framework requiring motor coordination and balance [33].

FKBP52$^{+/-}$ mice's impairments in motor coordination were independent of their locomotor activity since FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice covered a similar distance in the open field over thirty minutes, consistent with a previous report [7]. Whether FKBP52$^{+/-}$ mice showed decreased endurance [40] is unclear. However, they did not appear to display an altered motivation considering other tasks assessing exploratory drive, *i.e.* arm visits in the Y-maze or object exploration in the novel object tasks [23, 41] in which FKBP52$^{+/-}$ mice did not display a consistent distinct behaviour. Support for the observation of FKBP52 not being involved in processes controlling exploratory behaviour is further provided by a report of old

C57Bl/6N mice who showed a physiological age-dependent increase of cerebral FKBP52 levels but unaltered object exploration performance in a novel object recognition paradigm [11]. An intriguing observation of experiment 3 was that twelve-month-old female FKBP52$^{-/-}$ mice stayed on the accelerating rod for much longer than either FKBP52$^{+/+}$ or FKBP52$^{+/-}$ mice. This could perhaps be explained by their behaviour in the open field and Y-maze which pointed at a rather active phenotype [24, 27].

To date, altered motor function has not been reported as a feature of the FKBP52$^{+/-}$ phenotype [7]. **However, progressive deficits in motor coordination have been linked to neurodegeneration in a number of animals models [42-44], and FKBP52 was found to have a regenerative potential, promoting neuronal differentiation and neurite outgrowth in primary cell culture [45, 46], suggesting that downregulation of FKBP52 levels may lead to neurodegenerative changes to the brain.** In humans, FKBP52 has recently been investigated in diseases that present features of motor dysfunction: For example, low levels of FKBP52 have been measured in *post-mortem* brains obtained from patients diagnosed with frontotemporal dementia and parkinsonism linked to chromosome 17 [15]. On the other hand, high levels of FKBP52 were linked to increased α-synuclein aggregation – a key feature of Parkinson's disease pathology – *in vitro* [47]. These contradictory findings may therefore point towards a disease-dependent system in which FKBP52 is either ascribed protective or adverse properties. In both cases, FKBP52 has been suggested as a potential pharmaceutical target in the causative treatment of Alzheimer's [3] and Parkinson's disease [47, 48].

FKBP52's binding to glucocorticoid receptors is dependent on its exchange with FKBP51 [6]. Both proteins have theoretically been linked to mood disorders [49, 50]. **However, until now, pre-clinical studies have demonstrated a robust association of FKBP51 with stress response and depression-like behaviour [4, 49, 51-55], but** to the author's knowledge, no study has been published on FKBP52 and depression or another mood-disorder yet. However, FKBP52$^{+/-}$ mice appear to be more sensitive to stress, applied through chronic social defeat, than FKBP52$^{+/+}$ mice in some behavioural paradigms tested like in a sociability test [7]. Although, a more robust behaviour was also observed in stressed FKBP52$^{+/-}$ mice in other tasks, such as the latency to first floating in a forced swim test [7]. Unstressed mice of both genotypes appear to behave alike in a number of paradigms measuring emotionality-related parameters, such as the time struggling and floating in the forced swim test or relative time spent in the centre zone of an open field [7]. Matching those observations is the result of the

present study: FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice spent a similar amount of time in the centre of an open field; suggesting reduced FKBP52$^{+/-}$ levels in the mouse brain [7] were not in themselves reflected in an anxiety-related profile. The present observation of FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice spending a similar amount of time in the open arms of an elevated plus maze further supports this, is, however, in disagreement with a previous report [7]. One possible explanation is that mice in our study were subjected to elevated plus maze at the end of the behavioural battery, whereby repeated handling may have alleviated their anxiogenic-like behaviour.

FKBP52$^{+/-}$ further appeared to perform similarly to age- and sex-matched FKBP52$^{+/+}$ mice in terms of species-specific behaviour, *i.e.* food burrowing, and in terms of learning and memory in a spatial, object-oriented or fear context at all ages tested (from two- to eighteen-months-of-age) which has not been investigated before. It has, however, been shown that increased FKBP52 levels in aged C57Bl/6N mice were not associated with deficits in an object recognition task [11]. Thus, together with our findings, this rules out a role of FKBP52 in recognition memory.

The observation of female mice being lighter than male mice is consistent with previous reports of C57Bl/6J and Sv129 x C57BL/6J control mice [56, 57]. A steady increase in body mass from two- to eighteen-months-of-age irrespective of sex, as found in experiment 2, has been reported for C57Bl/6J and Sv129 x C57BL/6J control mice in the past [56, 57]. The amount of food burrowed declined with age, as shown previously in C57Bl/6J mice ranging from two- to twelve- [20] and from four- to twenty-one-months-of-age [58]. Similarly, locomotor activity has been shown to decrease with advancing age [59] like in the present study. The magnitude of the acoustic startle response also decreased with mouse age which seems to be consistent with a published report [60]. In the contextual fear paradigm of experiment 2, two-month-old male and female mice became similarly more and more immobile with increasing number of electric shocks applied to their feet; as found previously [48, 61].

5 Conclusion – Outlook

In the past, low levels of FKBP52 have been associated with high levels of tau in AD [15]. A causal relationship between FKBP52 and tau has also been demonstrated *in vitro* [14].

Furthermore, FKBP52 has been ascribed a modulatory role in Aβ-toxicity in a genetic construct of AD [12]. Whilst it has been shown that tau levels are predictive of cognitive decline in AD [13], it has not been tested whether FKBP52 in itself affects cognition or behaviour relevant to AD. The results of the present study, namely that FKBP52$^{+/-}$ mice appeared to be cognitively and behaviourally comparable to FKBP52$^{+/+}$ mice over a wide range of ages and across sexes – with the exception of motor coordination –, now confirmed **that reduced FKBP52 levels are not a risk factor to cognitive decline as seen in AD**. This is in keeping with a previous report that found no relationship between object memory and cerebral FKBP52 levels [11]. Therefore, FKBP52 is more likely to play a modulatory role in the pathology of AD, **but the motor coordination deficits associated with reduced FKBP52 levels suggest that this protein is neuroprotective in ageing.**

Conflict of Interest

None

Funding

This research was funded by the Fondation Nationale de Gerontologie, France, and the Institut Baulieu, France.

Acknowledgements

The authors express their gratitude to Stacey Knapp and Matthew Dawes for their assistance in acquiring the behavioural data. Thanks are due to Béatrice Chambraud and Étienne-Émile Baulieu at the Institut National de la Santé et de la Recherche Médicale Unité Mixte de Recherche 788, Université Paris XI, Kremlin Bicêtre 94276, France, for helpful comments.

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Figures legends

Figure 1. Order of behavioural and cognitive tests FKBP52$^{+/+}$, FKBP52$^{+/-}$ mice and FKBP52$^{-/-}$ mice underwent over fifteen days in **(1 A)** experiment 1 and **(1 B)** experiments 2 and 3. Experiment 1 was characterised by the re-use of FKBP52$^{+/+}$, FKBP52$^{+/-}$ mice at twelve-months-of-age after initial testing at ten-months-of-age. Since contextual fear conditioning and elevated plus maze testing (indicated by *) are not repeatable [62], experiment 1 mice were subjected to these two tests at twelve-months-of-age only. Experiment 2 covered an age-range from two- to eighteen-months-of-age in experimentally naïve FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice; whilst experiment 3 saw the addition of FKBP52$^{-/-}$ mice at twelve-months-of-age.

Figure 2. Body mass measurements recorded for male and female FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice at different ages in three separate experiments. Whilst female FKBP52$^{+/-}$ mice weighed more than FKBP52$^{+/-}$ mice in experiment 1 (2 A), this could not be confirmed by data obtained from experiment 2 (2 B) or 3 (2 C). KEY: *F* female, M male; ** $p < 0.01$ (ANOVA/mixed model *post-hoc* planned comparison).

Figure 3. Number of arms visited in a Y-maze by female and male FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice at different ages in three separate experiments. Statistical analysis of experiment 2 flagged up a genotype-related difference in this parameter which was, however, only significant between fifteen-month-old female FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice (3 B). KEY: *F* female, M male; ** $p < 0.01$ (ANOVA/mixed model *post-hoc* planned comparison).

Figure 4. Total distance moved in the open field by male and female FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice at different ages in three separate experiments. Whilst FKBP52$^{+/-}$ mice did not differ from FKBP52$^{+/+}$ mice in terms of locomotor activity (4 A and 4 B); experiment 3 (4 C) highlighted that female FKBP52$^{-/-}$ mice covered a greater distance than female FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice – reaching locomotor activity levels of male FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice. The opposite was observed for male FKBP52$^{-/-}$ mice. KEY: *F* female, M male.

Figure 5. Preference indices within the novel object discrimination paradigm: For male and female FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice at different ages in three separate experiments. Repeatedly tested twelve-month-old female FKBP52$^{+/+}$ mice spent more time exploring a novel object than their FKBP52$^{+/-}$ littermates **(5 A). This observation was not robust when explored in naïvely tested mice of experiment 2 (5 B) and 3 (5 C).** KEY: *F* female, M male; * $p < 0.05$ (ANOVA/mixed model *post-hoc* planned comparison), and # $p < 0.05$ (one-sample *t*-test; comparison of means to 50 %).

Figure 6. Accelerod performance in the longitudinal experiment 1 testing the latency to fall from an accelerating rotating rod of (6 A) ten- and (6 B) twelve-month-old females, as well as (6 E) ten- and (6 F) twelve-month-old male FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice. Figures (6 C) and (6 G) illustrate the change in performance from ten- to twelve months in female and male mice, respectively. FKBP52$^{+/-}$ mice remained on the rods for shorter than FKBP52$^{+/+}$ mice which was more obvious in repeatedly tested mice at twelve-months-of-age. (6 D, 6 H) Accelerod performance in experiment 3 of female (6 D) and male (6 H) FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice; whereby latency to fall from the rod is shown. Experimentally naïve twelve-month-old FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice performed similarly; but female FKBP52$^{-/-}$ mice remained on the rod for longer than female FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice. This may be explained by their increased locomotor activity measured in the open field. KEY: * $p < 0.05$, ** $p < 0.01$ (ANCOVA/mixed model *post-hoc* planned comparison).

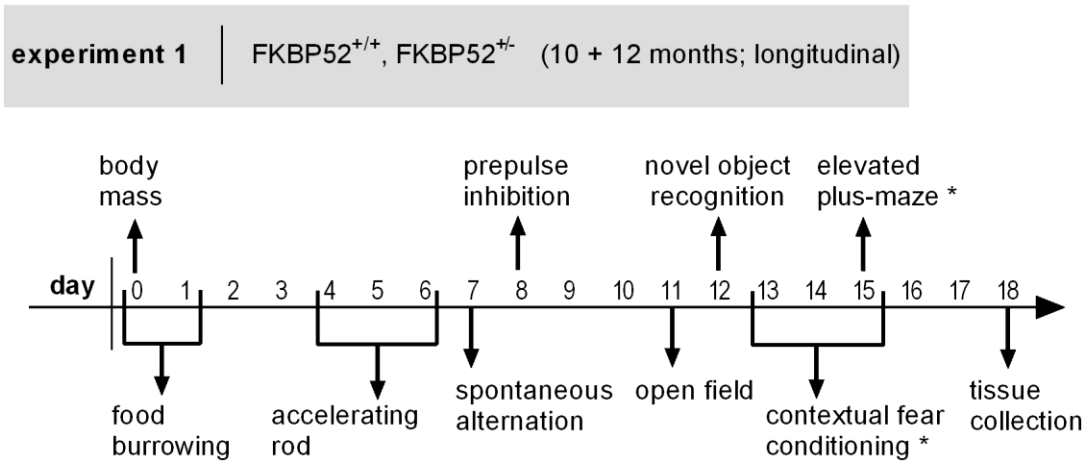
Figure 7. Accelerod performance in the cross-sectional experiment 2 of two- (6 A, 6 E), twelve- (6 B, 6 F), fifteen- (6 C, 6 G) and eighteen-month-old (6 D, 6 H) female (6 A-D) and male (6 E-H) FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice; whereby latency to fall from the rod is shown. Overall, experimentally naïve FKBP52$^{+/-}$ mice appeared to perform worse on the accelerating rotating rod than age- and sex-matched FKBP52$^{+/+}$ mice; however, at age- and sex- level

these differences seemed small and punctual. KEY: * $p < 0.05$ (ANCOVA/mixed model *post-hoc* planned comparison).

Figure 8. Latency of acoustic startle response of male and female FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice at different ages in three separate experiments. Statistical analysis pointed towards an overall genotype-effect of the latency of an acoustic startle response in experiment 1 only (8 A). This trend did not reach significance at age- or sex-level. KEY: *F* female, M male.

Figure 9. Preference for open arms of the elevated plus-maze by male and female FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice at different ages in three separate experiments. The genotypes did not differ from each other in the time the mice spent on the open arm of an elevated plus maze; except between eighteen-month-old FKBP52$^{+/-}$ and FKBP52$^{+/+}$ mice of experiment 2 **(9 B).** KEY: *F* female, M male; * $p < 0.05$ (ANOVA/mixed model *post-hoc* planned comparison).

A



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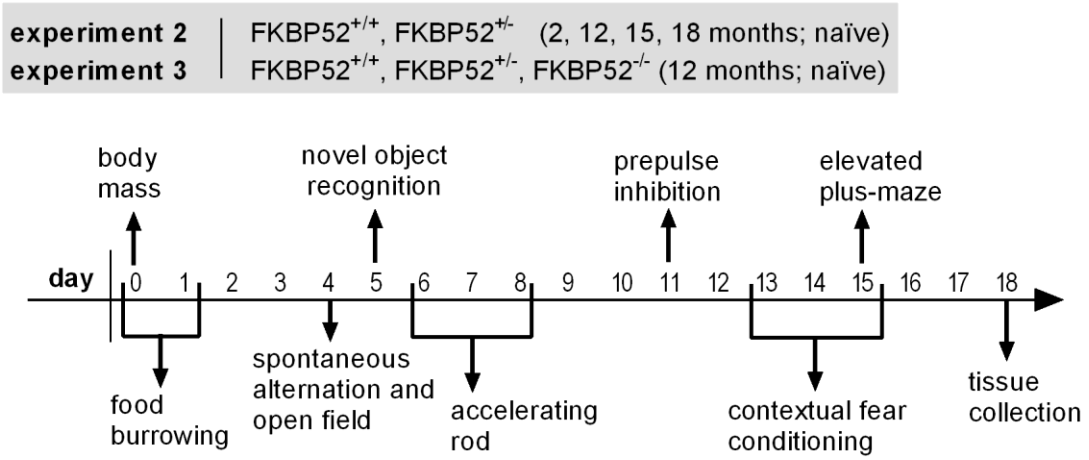


Fig. 1

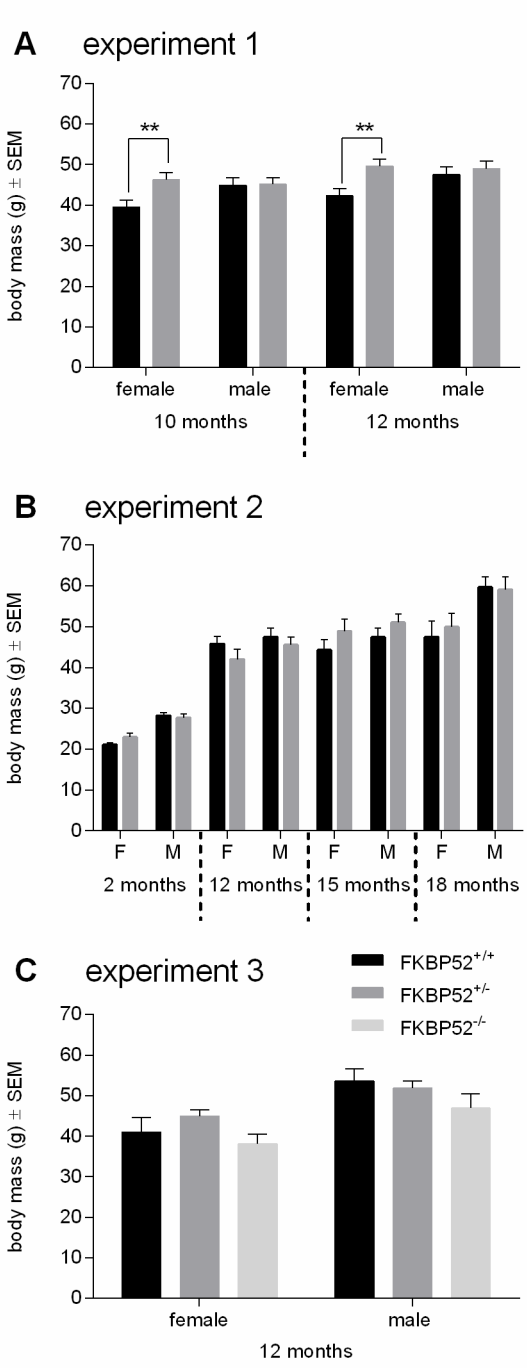


Fig. 2

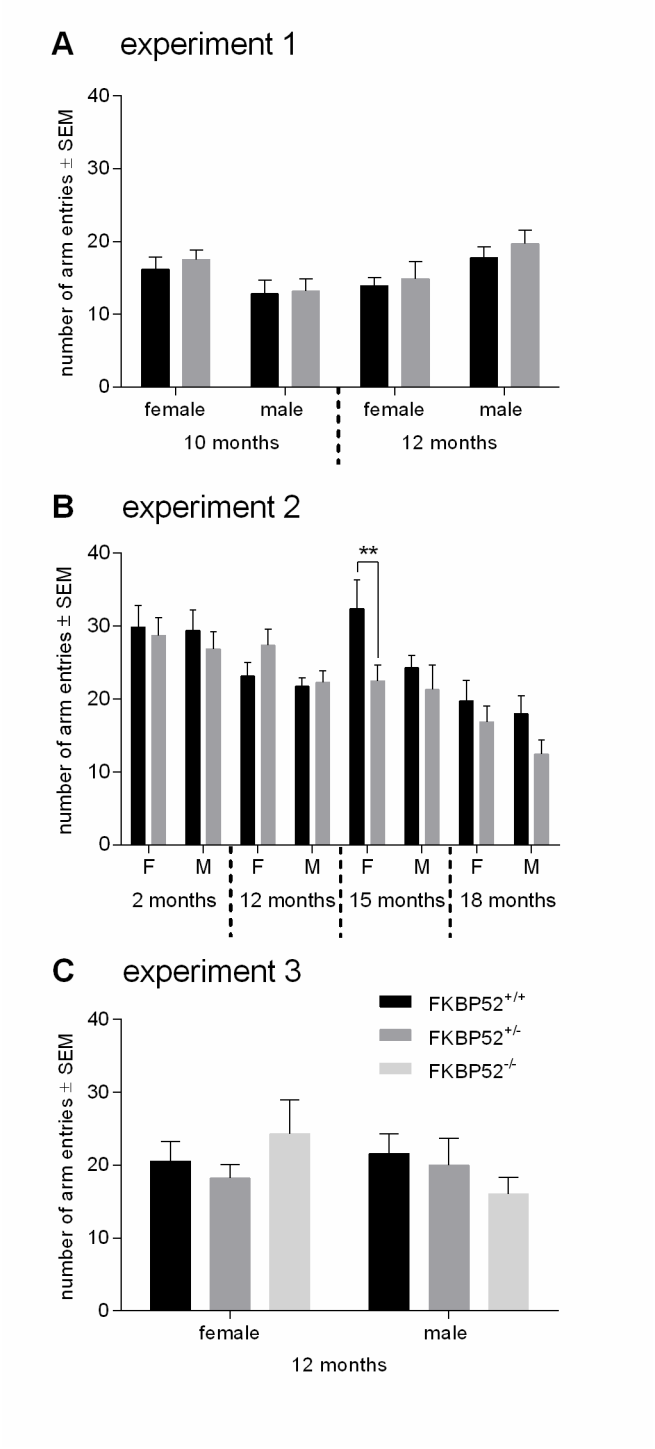


Fig. 3

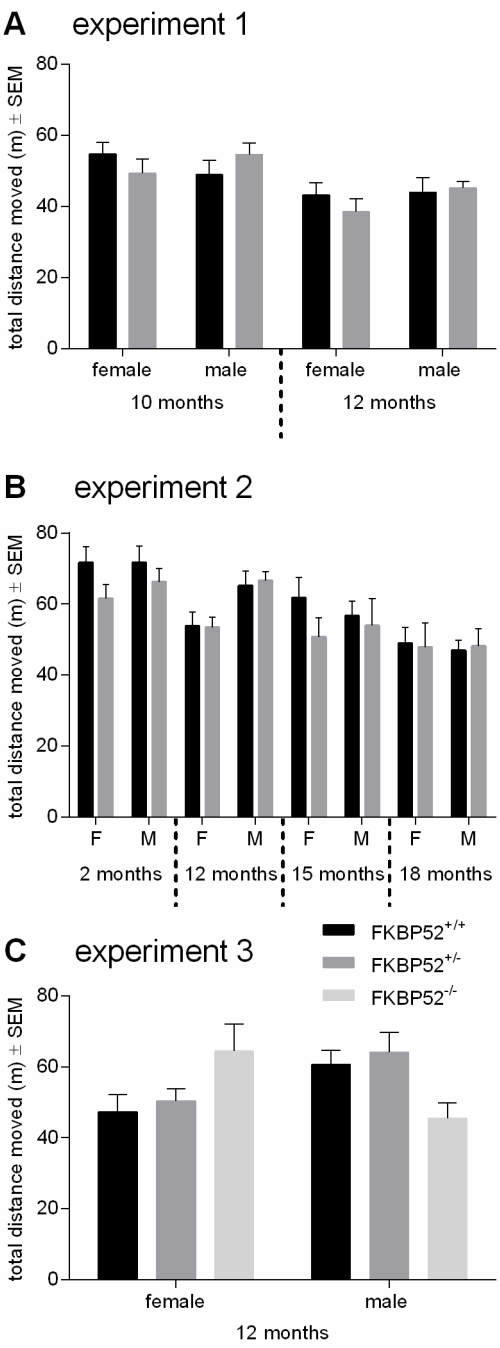


Fig. 4

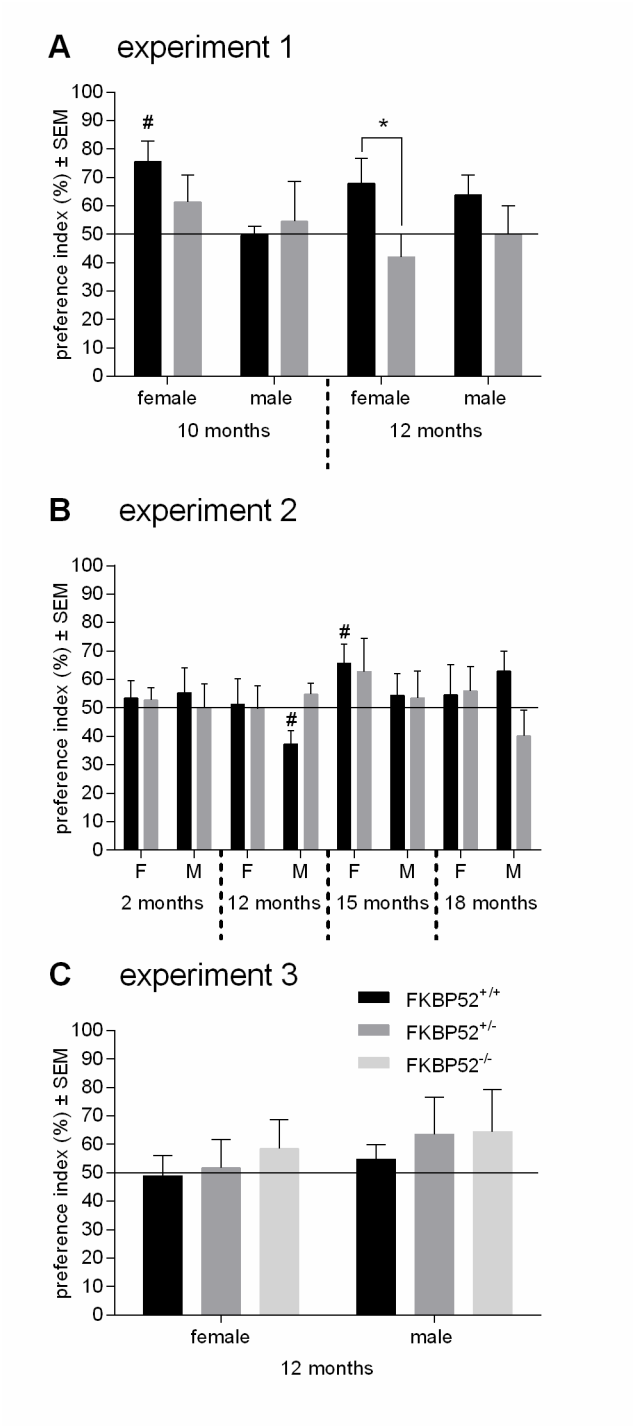


Fig. 5

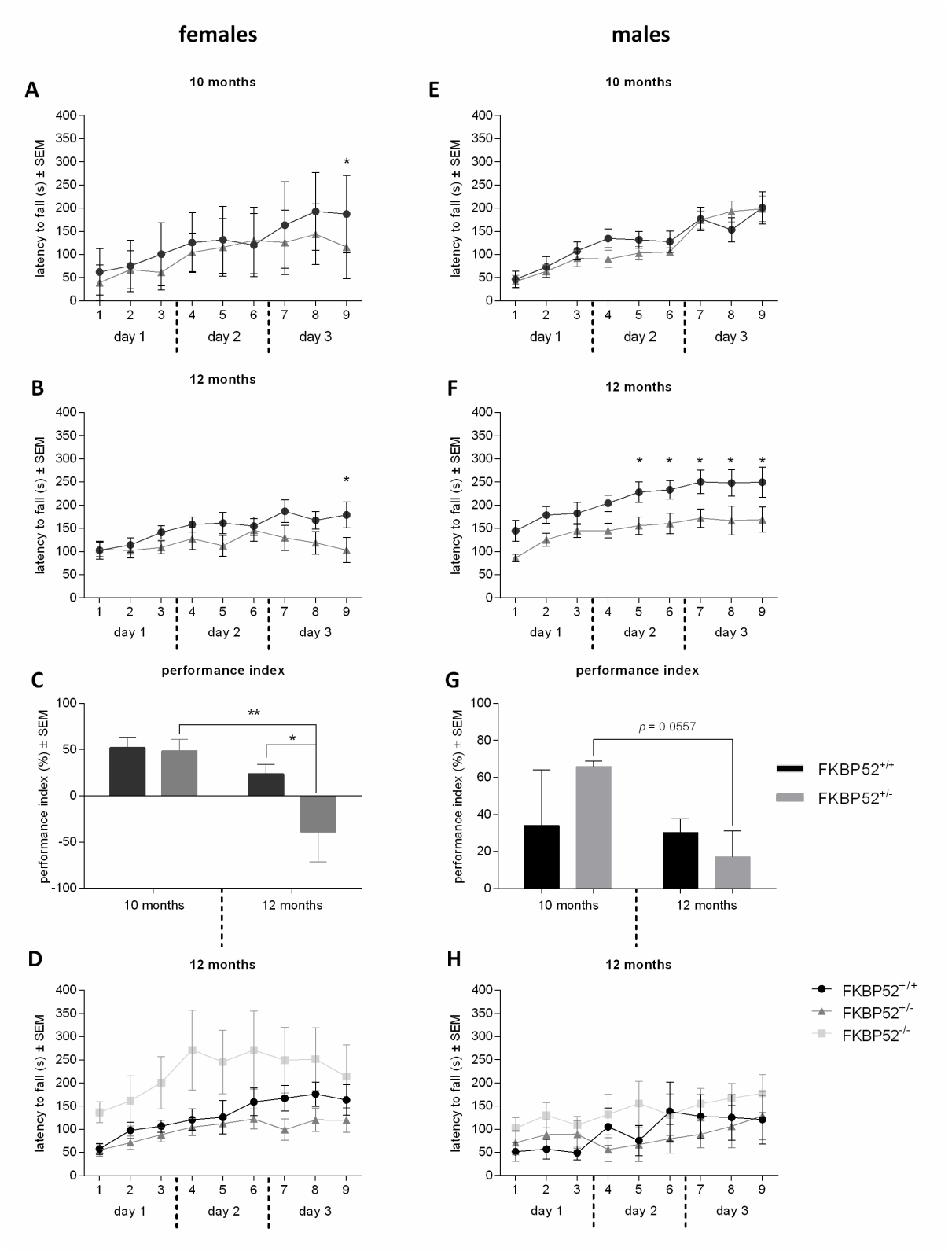


Fig. 6

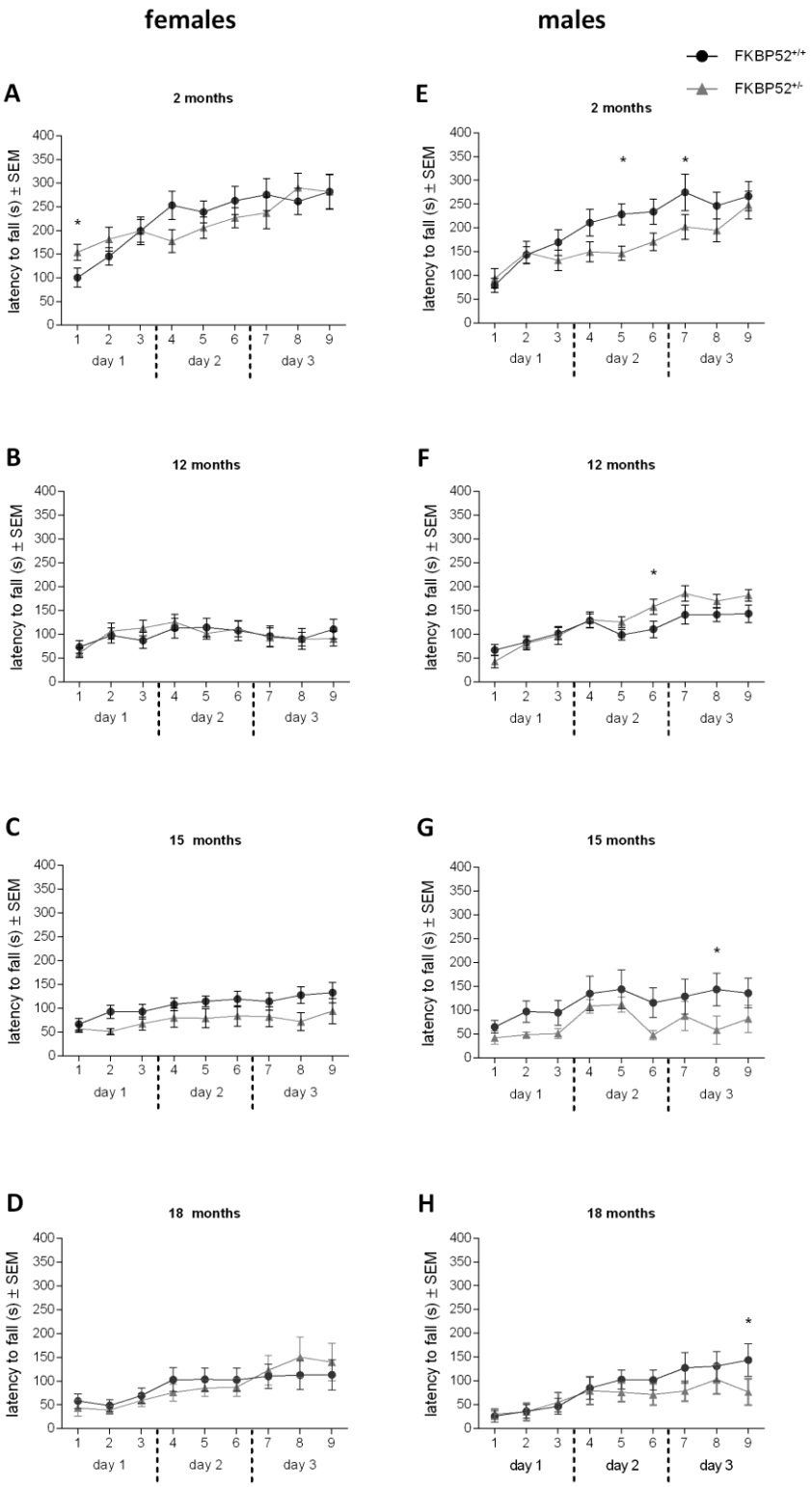


Fig. 7

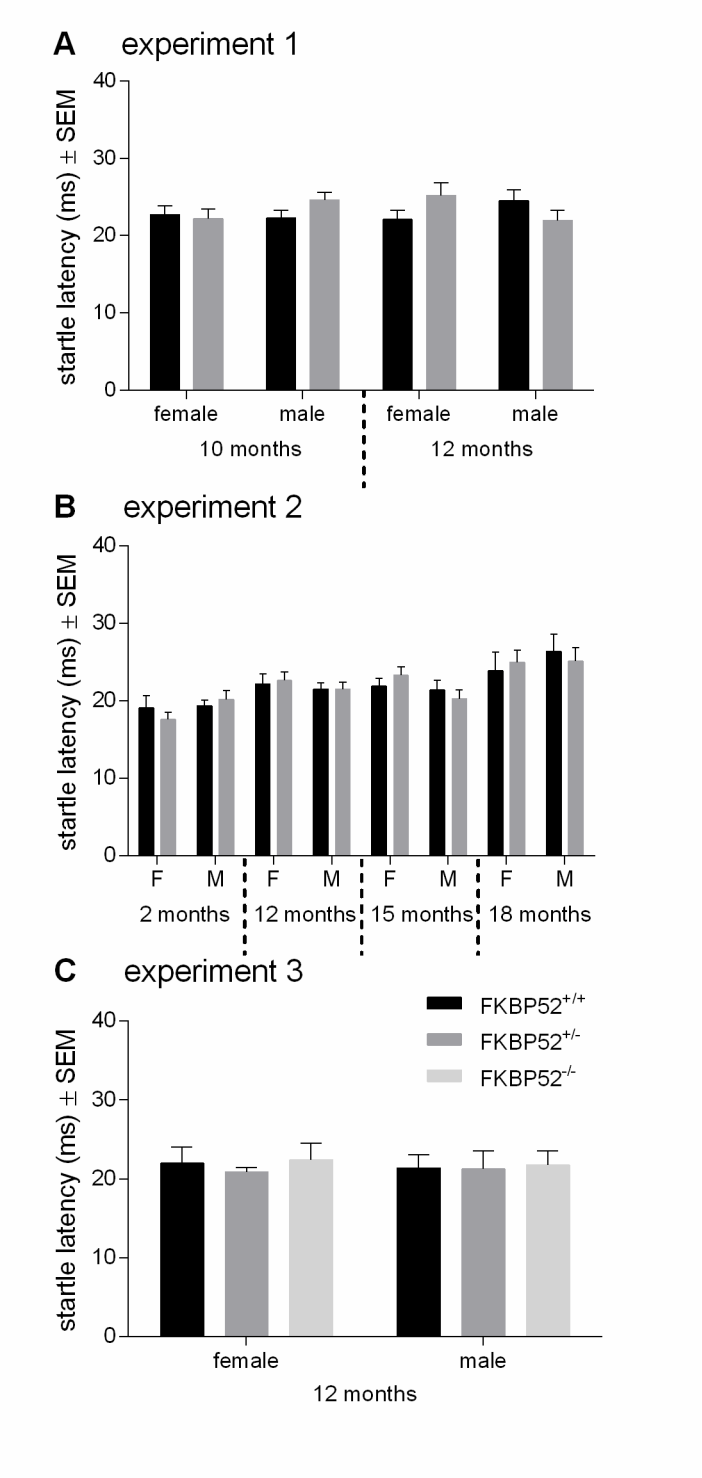


Fig. 8

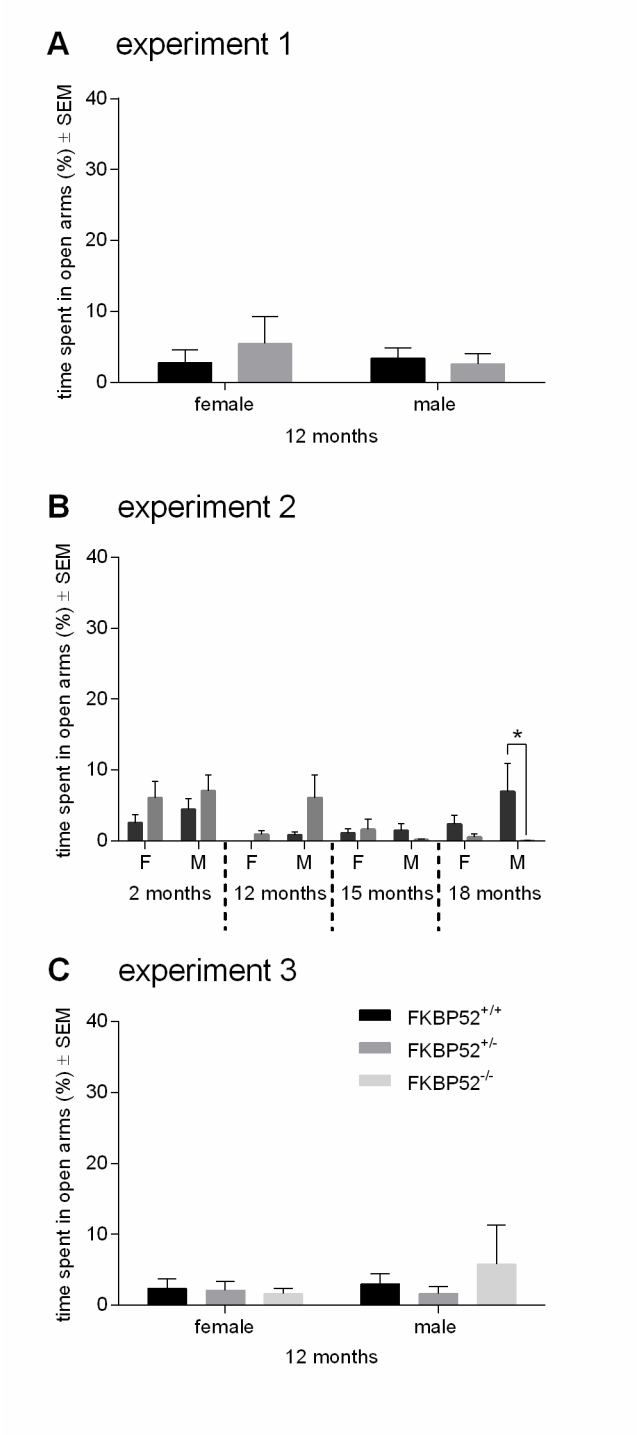


Fig. 9

Caption to figure 1: Number of mice used in experiments 1, 2 and 3.

experiment	**age**	**genotype**					
	(months)	**FKBP52$^{+/+}$**		**FKBP52$^{+/-}$**		**FKBP52$^{-/-}$**	
		female	**male**	**female**	**male**	**female**	**male***
1	**10 and 12**	12	12	12	11	-	-
2	**2**	12	12	12	12	-	-
	12	12	12	12	12	-	-
	15	10	9	10	6	-	-
	18	9	10	8	9	-	-
3	**12**	**11**	**10**	**5**	**5**	**6**	**5**

* some FKBP52$^{-/-}$ mice displayed intersex characteristics externally presented with under-developed testicles upon dissection. Female FKBP52$^{-/-}$ mice appeared inconspicuous.

Caption to table 2: ANOVA results of body mass, behavioural and cognitive tests that indicated a significant effect of FKBP52 knock-down in experiment 1 and 2. Summary of main findings in experiment 3. KEY: df degree(s) of freedom, R residuals, F F-value, *p* *p*-value.

	test	**parameter**	**effect**	**df**	**R**	**F**	***p***	**comment**
experiment 1	body mass	body mass	genotype	1	43	5.28	0.0265	female $FKBP52^{+/-}$ heavier than $FKBP52^{+/+}$
	novel object	preference for novel object	genotype	1	41	4.34	0.0435	12-month-old female $FKBP52^{+/-}$ explored novel object less than $FKBP52^{+/+}$
	accelerod	performance index	genotype x age	1	33	4.25	0.0472	$FKBP52^{+/-}$'s latency to fall decreased with age
		latency to fall at 10 months	genotype x sex x trial	8	272	2.02	0.0441	female $FKBP52^{+/-}$ fell off the rod before $FKBP52^{+/+}$
		latency to fall at 12 months	genotype	1	33	5.29	0.0279	$FKBP52^{+/-}$ fell off the rod before $FKBP52^{+/+}$
	acoustic startle response	startle latency	genotype x age x sex	1	42	6.47	0.0147	not significant at age level
experiment 2	spontaneous alternation	number of arms entered	genotype x age	3	151	2.99	0.033	15-month-old female $FKBP52^{+/-}$ visited less arms than $FKBP52^{+/+}$
	accelerod	latency to fall	genotype x age x trial	24	1200	2.26	0.0005	$FKBP52^{+/-}$ fell off the rod before $FKBP52^{+/+}$

	elevated plus-maze	relative time spent in open arm	genotype x age	3	147	4.08	0.0081	18-month-old male $FKBP52^{+/-}$ less time in open arms than $FKBP52^{+/+}$
experiment 3	open field	distance moved	female $FKBP52^{-/-}$ moved more than $FKBP52^{+/-}$ and $FKBP52^{+/+}$; male (inter-sex) $FKBP52^{-/-}$ moved less than male $FKBP52^{+/-}$ and $FKBP52^{+/+}$					
	accelerod	latency to fall	female $FKBP52^{-/-}$ stayed on the rod for longer than $FKBP52^{+/-}$ and $FKBP52^{+/+}$					

Supplementary Material

1 Statistical results

All AN(C)OVA and mixed model results obtained from body mass, behavioural and cognitive measurements that were recorded in experiment 1 and 2 are summarised in Supplementary Tables 1 and 2, respectively. All results that demonstrate a main effect of genotype or its interaction with mouse sex or age are discussed in the main article. All other results are described in conjunction with the Supplementary Figures in the following.

2 Supplementary tables

Supplementary Table T1. AN(C)OVA results from body mass measurements and behavioural and cognitive performances recorded in longitudinal experiment 1 comprising ten- and twelve-month-old FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice. KEY: df degrees of freedom; R residuals.

TEST	PARAMETER	effect	df	R	F-value	*p*-value
body mass	mass	**genotype**	**1**	**43**	**5.28**	**0.0265**
		age	**1**	**43**	**101.67**	**< 0.0010**
		sex	1	43	1.78	0.1891
		genotype x sex	1	43	3.3	0.0761
		genotype x age	1	43	1.89	0.1762
		sex x age	1	43	0.05	0.8293
		genotype x age x sex	1	43	0.17	0.68
food burrowing	% food displaced	genotype	1	43	2.52	0.1198
		age	1	42	3.21	0.0804
		sex	1	43	0	0.989
		genotype x sex	1	43	0.53	0.472
		genotype x age	1	42	3.52	0.0678
		sex x age	1	42	1.81	0.186
		genotype x age x sex	1	42	0.12	0.7289
		body weight	**1**	**42**	**6.2**	**0.0168**
spontaneous alternation	% alternation	genotype	1	43	0.06	0.8087
		age	1	43	0.18	0.6728
		sex	1	43	0.23	0.6344
		genotype x sex	1	43	0.16	0.6925
		genotype x age	1	43	1.1	0.3003
		sex x age	1	43	3.03	0.0887
		genotype x age x sex	1	43	0.14	0.709
	number of arms entered	genotype	1	43	0.75	0.3908
		age	1	43	2.04	0.1608
		sex	1	43	0.03	0.8537
		genotype x sex	1	43	0	0.9989
		genotype x age	1	43	0.04	0.8375
		sex x age	**1**	**43**	**13.92**	**0.0006**
		genotype x age x sex	1	43	0.2	0.6548
open field	total distance moved	genotype	1	43	0.11	0.7395
		age	**1**	**43**	**17.76**	**0.0001**
		sex	1	43	0.35	0.5546
		genotype x sex	1	43	2.41	0.1279
		genotype x age	1	43	0.18	0.6761
		sex x age	1	43	0.85	0.3628
		genotype x age x sex	1	43	0.33	0.5679

	% distance moved in centre	genotype	1	43	0.22	0.6377
		age	1	43	0.48	0.4899
		sex	1	43	0	0.9948
		genotype x sex	1	43	1.71	0.1978
		genotype x age	1	43	0	0.9795
		sex x age	1	43	0	0.9838
		genotype x age x sex	1	43	0.48	0.4921
novel object						
- **habituation**	total exploration time	genotype	1	43	0	0.9721
		age	1	43	0.51	0.4827
		sex	1	43	2.8	0.1018
		genotype x sex	1	43	0.04	0.8494
		genotype x age	1	43	0.47	0.5006
		sex x age	1	43	2.07	0.1684
		genotype x age x sex	1	43	0.95	0.3441
- **location**	total exploration time	genotype	1	43	0.02	0.8796
		age	1	43	0.34	0.5667
		sex	1	43	0.56	0.4596
		genotype x sex	1	43	1.67	0.2032
		genotype x age	1	43	0.1	0.758
		sex x age	1	43	0.03	0.8581
		genotype x age x sex	1	43	2.44	0.1393
	preference index	genotype	1	43	1.04	0.3131
		age	1	43	3.28	0.0902
		sex	1	43	0.02	0.8978
		genotype x sex	1	43	0.88	0.3531
		genotype x age	1	43	0.03	0.8613
		sex x age	1	43	1.14	0.3031
		genotype x age x sex	1	43	1.7	0.212
- **discrimination**	total exploration time	genotype	1	43	0.19	0.6658
		age	1	43	2.07	0.1683
		sex	1	43	4.64	**0.0371**
		genotype x sex	1	43	0.55	0.4645
		genotype x age	1	43	0.01	0.9069
		sex x age	1	43	1.61	0.2218
		genotype x age x sex	1	43	2.73	0.1168
	preference index	**genotype**	1	43	4.34	**0.0435**
		age	1	43	0.55	0.5306
		sex	1	43	0.4	0.4677
		genotype x sex	1	43	0.97	0.3309
		genotype x age	1	43	1.8	0.1975
		sex x age	1	43	1.8	0.1974
		genotype x age x sex	1	43	0.09	0.7665
accelerod (at 10 months)	latency to fall	genotype	1	33	0.74	0.3947
		sex	1	33	0.46	0.5018

		trial	**8**	**272**	**38.6**	**< 0.0010**
		genotype x sex	1	33	0.18	0.671
		genotype x trial	8	272	0.6	0.7747
		sex x trial	8	272	1.81	0.0759
		genotype x sex x trial	**8**	**272**	**2.02**	**0.0441**
		body weight	1	33	1.45	0.2379
accelerod (at 12 months)	latency to fall	**genotype**	**1**	**33**	**5.29**	**0.0279**
		sex	**1**	**33**	**8.26**	**0.007**
		trial	**8**	**272**	**14.56**	**< 0.0010**
		genotype x sex	1	33	1.21	0.2792
		genotype x trial	8	272	1.8	0.0769
		sex x trial	8	272	1.72	0.0931
		genotype x sex x trial	8	272	0.67	0.7214
		body weight	1	33	2.76	0.106
accelerod	performance index	genotype	1	34	0.57	0.4568
		age	**1**	**33**	**10.8**	**0.0024**
		sex	1	34	1.57	0.2184
		genotype x sex	1	34	2.94	0.0953
		genotype x age	**1**	**33**	**4.25**	**0.0472**
		sex x age	1	33	1.6	0.2142
		body weight	1	33	0.59	0.4474
		genotype x age x sex	1	33	0.08	0.7828
acoustic startle response	startle latency	genotype	1	43	0.24	0.6289
		age	1	42	0.32	0.572
		sex	1	43	0.07	0.7903
		genotype x sex	1	43	0.51	0.4772
		genotype x age	1	42	0.11	0.7457
		sex x age	1	42	0.56	0.4566
		genotype x age x sex	**1**	**42**	**6.47**	**0.0147**
		body weight	1	42	0.41	0.5256
	startle magnitude	genotype	1	43	0.39	0.535
		age	**1**	**42**	**6.33**	**0.0158**
		sex	1	43	0.13	0.7166
		genotype x sex	1	43	0.04	0.8434
		genotype x age	1	42	0.14	0.7107
		sex x age	1	42	2.15	0.1503
		genotype x age x sex	1	42	0.06	0.8118
		body weight	**1**	**42**	**4.35**	**0.0432**
	% prepulse inhibition (at 10 months)	genotype	1	42	1.38	0.2475
		sex	**1**	**42**	**4.45**	**0.0409**
		dB level	**3**	**129**	**20.08**	**< 0.0010**
		genotype x sex	1	42	0.17	0.6845
		genotype x dB level	3	129	0.22	0.885
		sex x dB level	3	129	1.09	0.3557
		genotype x sex x dB	3	129	1.26	0.2919

		level				
		body weight	**1**	**42**	**10.26**	**0.0026**
	% prepulse inhibition (at 12 months)	genotype	1	42	0.06	0.8075
		sex	1	42	3.8	0.0578
		dB level	**3**	**129**	**8.71**	**< 0.0010**
		genotype x sex	1	42	2.91	0.0953
		genotype x dB level	3	129	0.75	0.5225
		sex x dB level	3	129	0.67	0.5707
		genotype x sex x dB level	3	129	0.24	0.8707
		body weight	**1**	**42**	**4.24**	**0.0458**
contextual fear (at 12 months)	time immobile (fear memory acquisition)	genotype	1	43	0.67	0.4174
		sex	**1**	**43**	**5.32**	**0.0259**
		shock	**9**	**387**	**66.06**	**< 0.0010**
		genotype x sex	1	43	0.11	0.7441
		genotype x shock	9	387	1.74	0.0785
		sex x shock	**9**	**387**	**3.64**	**0.0002**
		genotype x sex x shock	9	387	1.34	0.2165
	time immobile (fear memory retention)	genotype	1	43	0	0.9894
		sex	1	43	1.97	0.1672
		genotype x sex	1	43	0.73	0.3981
	time immobile (fear memory extinction)	genotype	1	43	0.09	0.7625
		sex	1	43	1.3	0.2599
		genotype x sex	1	43	0	0.9522
	extinction index	genotype	1	43	0.44	0.5109
		sex	1	43	0.6	0.4439
		genotype x sex	1	43	3.2	0.0809
elevated plus-maze (at 12 months)	% Time spent in open arms	genotype	1	40	0.72	0.4018
		sex	1	40	0.35	0.5547
		genotype x sex	1	40	0.16	0.6955

Supplementary Table T2. AN(C)OVA results from body mass measurements and behavioural and cognitive performances recorded in experiment 2 comprising experimentally naïve FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice at two-, twelve-, fifteen- and eighteen-months-of-age. KEY: df degrees of freedom; R residuals.

TEST	PARAMETER	Effect	df	R	F-value	*p*-value
body mass	mass	genotype	1	151	0.37	0.5435
		age	**3**	**151**	**136.05**	**<0.0001**
		sex	**1**	**151**	**29.11**	**<0.0001**
		genotype x age	3	151	1.19	0.3152
		age x sex	3	151	1.96	0.1227
		genotype x sex	1	151	0.04	0.8496
		genotype x age x sex	3	151	0.49	0.6931
food burrowing	% food burrowed	genotype	1	150	0.51	0.4753
		age	3	150	2.53	0.0594
		sex	1	150	0.97	0.3258
		genotype x age	3	150	2.01	0.1143
		age x sex	3	150	2.43	0.0671
		genotype x sex	1	150	2.03	0.1564
		genotype x age x sex	3	150	1.01	0.3898
		body weight	**1**	**150**	**5.22**	**0.0238**
spontaneous alternation	% alternation	genotype	1	151	1.02	0.3133
		age	3	151	0.74	0.5322
		sex	1	151	1.62	0.2054
		genotype x age	3	151	0.24	0.8664
		age x sex	3	151	0.82	0.4846
		genotype x sex	1	151	1.01	0.3154
		genotype x age x sex	3	151	1.41	0.2414
	number of arms entered	genotype	1	151	2.7	0.1026
		age	**3**	**151**	**15.7**	**<0.0001**
		sex	**1**	**151**	**4.96**	**0.0275**
		genotype x age	**3**	**151**	**2.99**	**0.033**
		age x sex	3	151	0.4	0.7563
		genotype x sex	1	151	0.01	0.915
		genotype x age x sex	3	151	0.72	0.5408
open field	total distance moved	genotype	1	151	1.81	0.18
		age	**3**	**151**	**13.78**	**<0.0001**
		sex	**1**	**151**	**5.86**	**0.0167**
		genotype x age	3	151	1.44	0.2332
		age x sex	**3**	**151**	**4.47**	**0.0049**
		genotype x sex	1	151	0.19	0.3741
		genotype x age x sex	3	151	0.11	0.9557
	% distance moved in	genotype	1	151	0.96	0.3285
		age	**3**	**151**	**9.46**	**<0.0001**

	centre	**sex**	**1**	**151**	**14.47**	**0.0002**
		genotype x age	3	151	0.86	0.4616
		age x sex	3	151	2.14	0.0976
		genotype x sex	1	151	1.97	0.1628
		genotype x age x sex	3	151	0.38	0.7642
novel object						
- **habituation**	total	genotype	1	140	0.59	0.4449
	exploration	age	3	140	0.46	0.7104
	time	sex	1	140	3.55	0.0617
		genotype x age	3	140	0.07	0.9751
		age x sex	3	140	0.19	0.9021
		genotype x sex	1	140	0	0.9819
		genotype x age x sex	3	140	1	0.3934
- **location**	total	genotype	1	142	1	0.3194
	exploration	age	3	142	0.08	0.9695
	time	sex	1	142	0.04	0.8466
		genotype x age	3	142	1.33	0.2674
		age x sex	3	142	0.86	0.4645
		genotype x sex	1	142	3.61	0.0596
		genotype x age x sex	3	142	0.14	0.9372
	preference	genotype	1	142	0.01	0.9428
	index	age	3	142	1.02	0.3858
		sex	1	142	0.48	0.4885
		genotype x age	3	142	2.03	0.1126
		age x sex	3	142	0.17	0.9175
		genotype x sex	1	142	0	0.984
		genotype x age x sex	3	142	2.2	0.0907
- **discrimination**	total	genotype	1	132	1.57	0.2123
	exploration	age	3	132	0.26	0.8570
	time	sex	1	132	0.12	0.7322
		genotype x age	3	132	0.52	0.6717
		age x sex	3	132	2.84	**0.0406**
		genotype x sex	1	132	0	0.9614
		genotype x age x sex	3	132	0.42	0.7418
	preference	genotype	1	132	0.08	0.7785
	index	age	3	132	1.42	0.2412
		sex	1	132	1.15	0.285
		genotype x age	3	132	1.1	0.3537
		age x sex	3	132	0.29	0.8327
		genotype x sex	1	132	0	0.9703
		genotype x age x sex	3	132	1.33	0.2662
accelerod	latency to fall	genotype	1	149	2.72	0.1009
		age	**3**	**149**	**2.98**	**0.0334**
		sex	1	149	1.76	0.1868
		trial	**8**	**1200**	**69.68**	**<0.0010**
		genotype x age	3	149	0.71	0.5495

		age x sex	3	149	2.09	0.1045
		age x trial	**24**	**1200**	**5.22**	**<0.0010**
		genotype x sex	1	149	0.69	0.4092
		genotype x trial	8	1200	1.81	0.0713
		sex x trial	**8**	1200	**2.42**	**0.0137**
		genotype x age x sex	3	149	0.81	0.491
		genotype x age x trial	**24**	**1200**	**2.26**	**0.0005**
		age x sex x trial	**24**	**1200**	**1.82**	**0.0089**
		genotype x sex x trial	8	1200	0.56	0.8077
		genotype x age x sex x trial	24	1200	1.16	0.2656
		body weight	**1**	**149**	**153.07**	**<0.0010**
acoustic startle response	startle latency	genotype	1	150	0.01	0.9317
		age	3	150	2.2	0.0899
		sex	1	150	0.46	0.5002
		genotype x age	3	150	0.08	0.9692
		age x sex	3	150	0.84	0.4721
		genotype x sex	1	150	0.1	0.7533
		genotype x age x sex	3	150	0.72	0.5396
		body weight	**1**	**150**	**4.33**	**0.0391**
	startle magnitude	genotype	1	150	0.93	0.3371
		age	**3**	**150**	**6.32**	**0.0005**
		sex	1	150	0.16	0.6933
		genotype x age	3	150	0.24	0.8714
		age x sex	3	150	0.21	0.8897
		genotype x sex	1	150	0.04	0.8501
		genotype x age x sex	3	150	0.36	0.7832
		body weight	**1**	**150**	**8**	**0.0053**
	% prepulse inhibition	genotype	1	150	0.35	0.5567
		age	**3**	**150**	**6.39**	**0.0004**
		sex	1	150	0	0.9463
		dB level	**3**	**453**	**177.37**	**<0.0010**
		age x genotype	3	150	0.51	0.6787
		age x sex	3	150	0.64	0.5917
		age x dB level	**9**	**453**	**4.41**	**<0.0010**
		genotype x sex	1	150	0.01	0.9214
		genotype x dB level	3	453	0.82	0.4813
		sex x dB level	3	453	0.64	0.5926
		genotype x age x sex	3	150	2	0.1163
		age x genotype x dB level	9	453	1.44	0.1689
		age x sex x dB level	9	453	1.12	0.3485
		genotype x sex x dB level	3	453	0.29	0.8304
		age x genotype x sex	9	453	0.39	0.9416

		x dB level				
		body weight	1	150	0.76	0.3833
contextual fear conditioning	time immobile (fear memory acquisition)	genotype	1	149	0.91	0.3422
		age	**3**	**149**	**6.06**	**0.0006**
		sex	**1**	**149**	**6.7**	**0.0106**
		shock	**9**	**1341**	**209.13**	**<0.0010**
		genotype x age	3	149	1.29	0.2812
		age x sex	3	149	2.12	0.0997
		age x shock	**27**	**1341**	**2.33**	**0.0001**
		genotype x sex	1	149	0.64	0.4253
		genotype x shock	9	1341	0.93	0.4934
		sex x shock	**9**	**1341**	**2.93**	**0.0019**
		genotype x age x sex	3	149	0.81	0.4898
		genotype x age x shock	27	1341	1.15	0.2702
		age x sex x shock	**27**	**1341**	**1.83**	**0.0058**
		genotype x sex x shock	9	1341	0.73	0.6794
		genotype x age x sex x shock	27	1341	0.6	0.9464
	time immobile (fear memory retention)	genotype	1	148	0	0.9639
		age	3	148	1.59	0.1931
		sex	**1**	**148**	**11.84**	**0.0008**
		genotype x age	3	148	1.49	0.219
		age x sex	**3**	**148**	**5.72**	**0.001**
		genotype x sex	1	148	0.33	0.5662
		genotype x age x sex	3	148	0.59	0.625
	time immobile (fear Memory extinction)	genotype	1	148	1.02	0.3134
		age	**3**	**148**	**3.92**	**0.01**
		sex	**1**	**148**	**21.3**	**< 0.0001**
		genotype x age	3	148	0.79	0.5037
		age x sex	**3**	**148**	**7.65**	**< 0.0001**
		genotype x sex	1	148	1.36	0.2447
		genotype x age x sex	3	148	0.99	0.3977
	extinction index	genotype	1	148	1.54	0.2166
		age	3	148	0.62	0.6057
		sex	1	148	1	0.3194
		genotype x age	3	148	1.64	0.1835
		age x sex	3	148	0.68	0.5652
		genotype x sex	1	148	0.46	0.4979
		genotype x age x sex	3	148	0.28	0.8426
elevated plus-maze	% time spent in open arms	genotype	1	147	0.28	0.5986
		age	**3**	**147**	**7.89**	**< 0.0001**
		sex	**1**	**147**	**5.61**	**0.0192**
		genotype x age	**3**	**147**	**4.08**	**0.0081**

	age x sex	3	147	1.37	0.2546
	genotype x sex	1	147	0.69	0.4084
	genotype x age x sex	3	147	0.23	0.8788

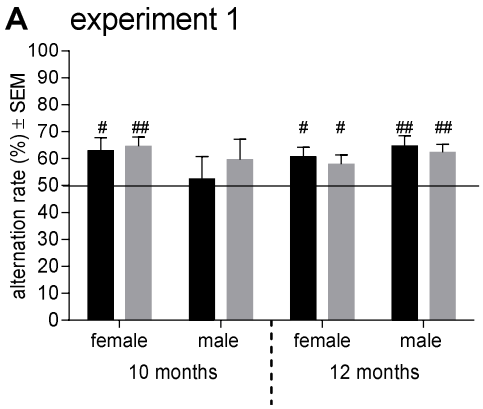
3 Supplementary figures: age- and sex-related results; irrespective of the FKBP52 genotype

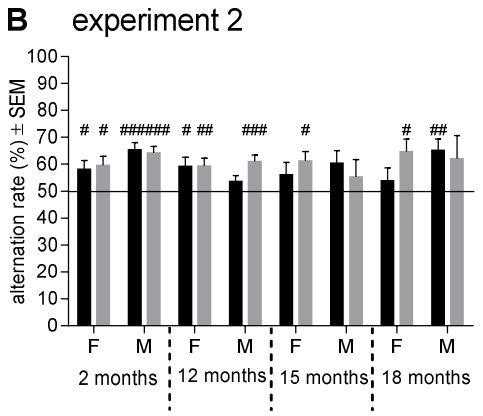
3.1 Food burrowing

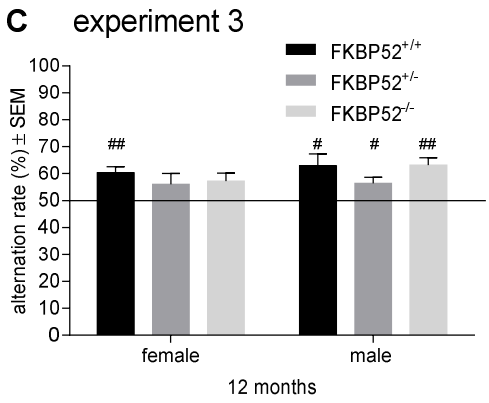
Supplementary Figure S1. Percentage of food displaced by male and female FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice at different ages in three separate experiments. Irrespective of sex and genotype, mice tended to displace less food from a jar with increasing age – from two- to eighteen-months-of-age – in experiment 2 (age: $F_{(3,\ 150)} = 2.53$, $p = 0.0594$; **S1 B**). Food burrowing behaviour was unaltered by any of the experimental conditions, *i.e.* age, sex or genotype, in the other two experiments **(S1 A, C)**.

. KEY: M male, F female.

3.2 Spontaneous alternation – alternation rate

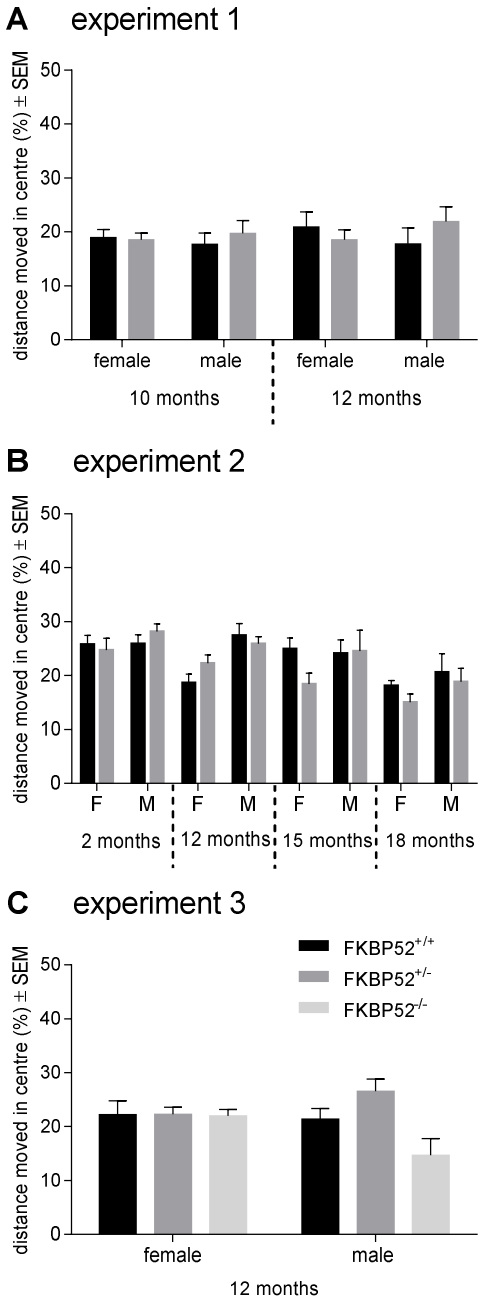






Supplementary Figure S2. Comparable alternation rates of male and female FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice in a Y-maze, at different ages in three separate experiments. Most mouse groups alternated above the 50%-chance level, except ten-month-old male mice in experiment 1 **(S2 A)**, fifteen-month-old male mice in experiment 2 **(S2 B)** and twelve-month-old female mice in experiment 3 **(S2 C)**. No group alternated at less than 50%. KEY: M male, F female; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ (one-sample *t*-test, comparison of means to 50%).

3.3 Open field – relative distance in the centre

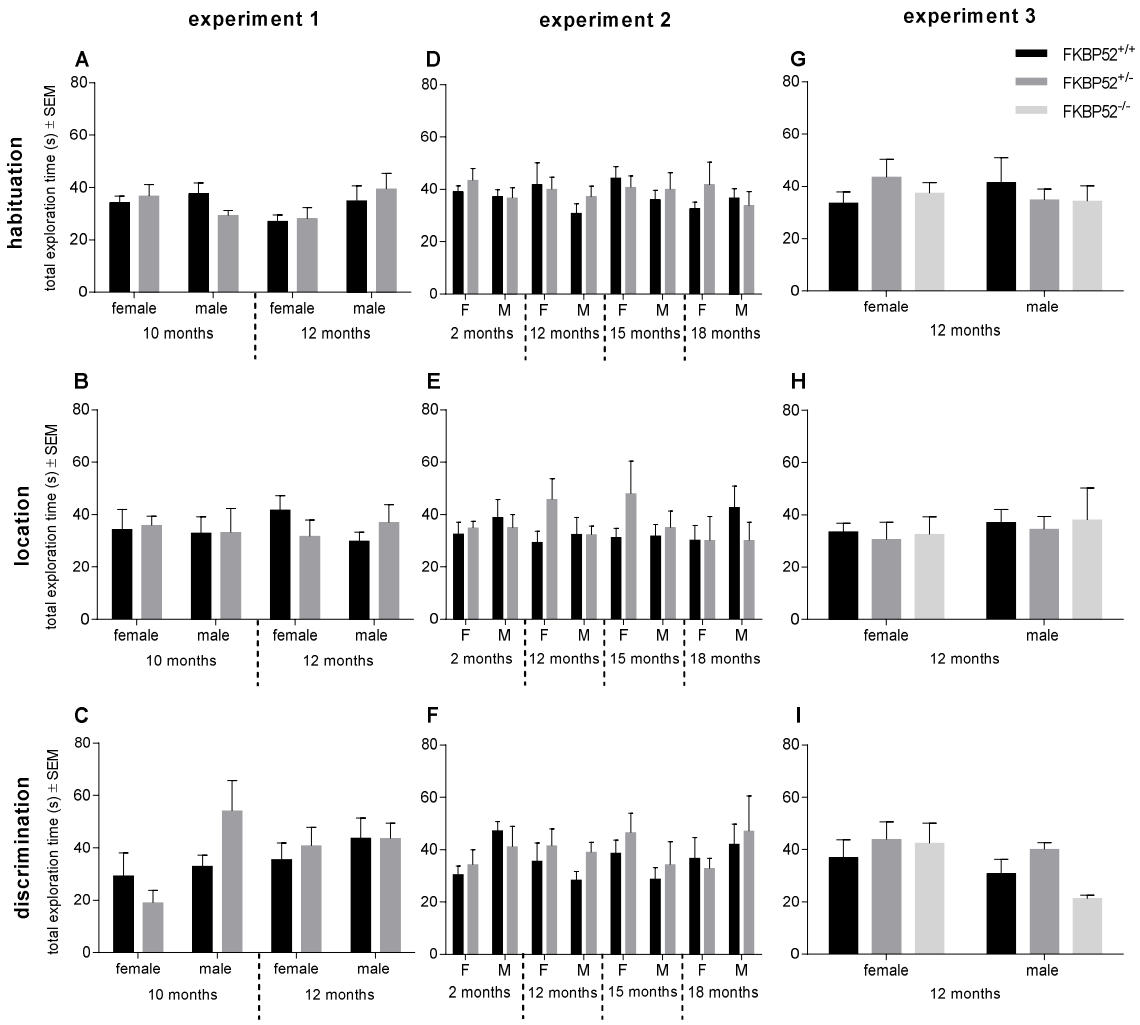


Supplementary Figure S3. The relative distance that male and female FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice covered in the centre of the open field arena declined from two- to eighteen-months-of-age (age: $F_{(3, 151)} = 9.46$, $p < 0.0001$) and varied with sex (sex: $F_{(1, 151)} = 14.47$, $p = 0.0002$) in experiment 2 **(S3 B)**. This variable was unaffected by the experimental conditions in experiment 1 **(S3 B)**. In experiment 3, male FKBP52$^{+/-}$ mice appeared to cover a greater, whereas male FKBP52$^{-/-}$ mice seemed to cover a shorter distance in the centre of the open

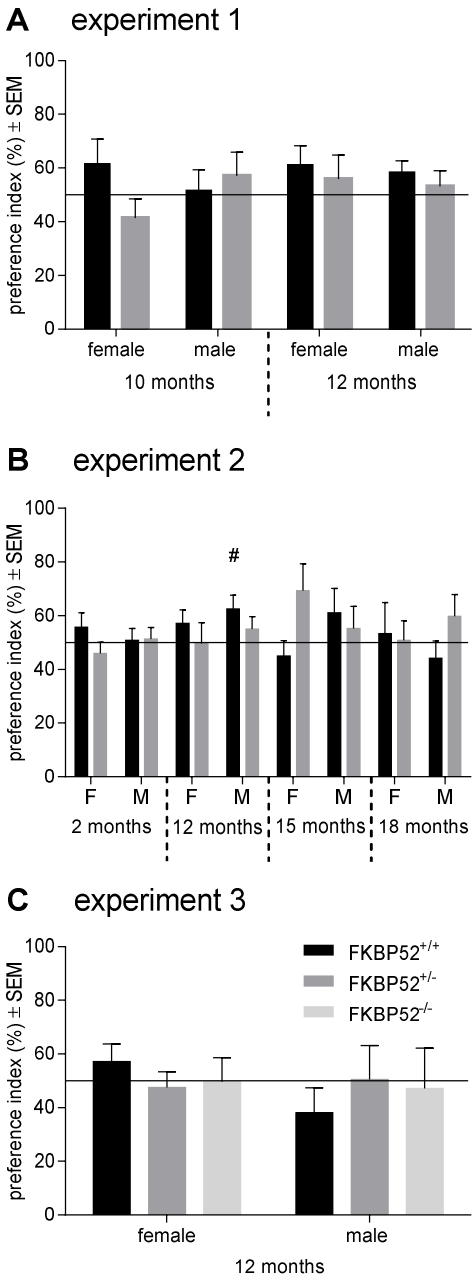
field than male FKBP52$^{+/+}$ mice **(S3 C)**. This observation was not supported by the statistics of data collected from experiment 1 or 2.

KEY: M male, F female.

3.4 Novel object habituation, location and discrimination

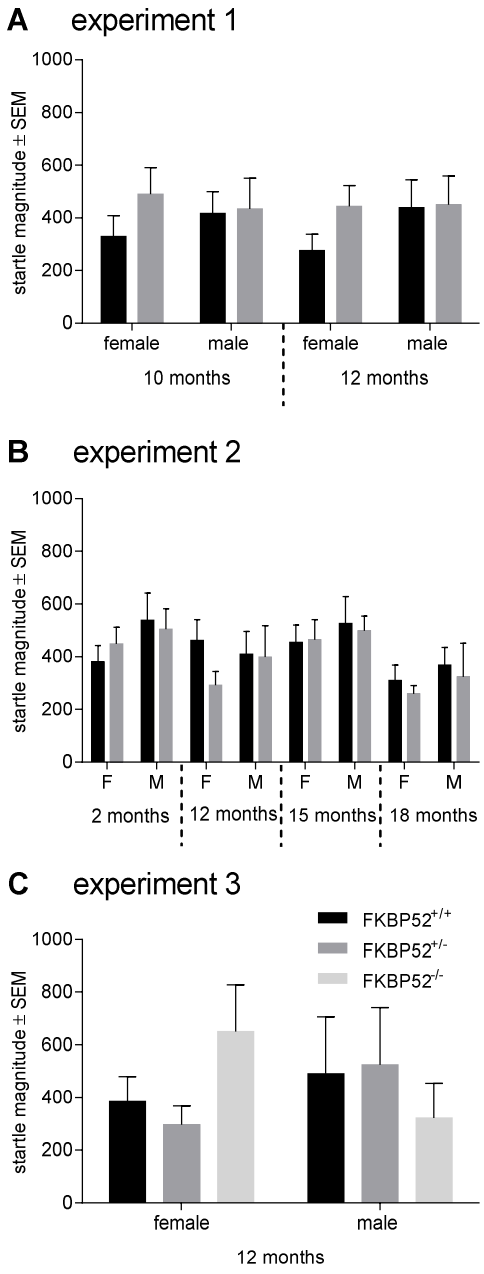


Supplementary Figure S4. Total exploration times of two objects did not differ between genotypes across all three novel object trials when male and female FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice were tested at different ages in three separate experiments. The time the mice spent exploring both objects within the habituation and location trials was not affected by the experimental conditions in experiment 1 and 2 **(S4 A, 4 B, 4 D and 4 E)**. The total exploration time of both objects in the discrimination trial depended on sex alone in experiment 1 (sex: $F_{(1, 43)} = 4.64$, $p = 0.0371$; **S4 C**) and on sex in an age-dependent manner in experiment 2 (sex x age: $F_{(3, 132)} = 2.84$, $p = 0.0406$; **S4 F**); but sex differences did not follow a coherent pattern. KEY: M male, F female.



Supplementary Figure S5. Whether mice preferred to explore an object at a new or familiar location was unaltered by genotype, sex or age in experiment 1, 2 and 3 (**S5 A, B** and **C**, respectively); covering FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice from two- to eighteen-months-of-age **(S5 B)** and including effects of repeated testing **(S5 A)**. The lack of genotype-related deficits may have arisen from poor FKBP52$^{+/+}$ performance which can currently not be explained as the testes were performed according to a previously validated protocol (Scullion et al., 2009).KEY: M male, F female; # $p < 0.05$ (one-sample *t*-test, comparison of means to 50 %).

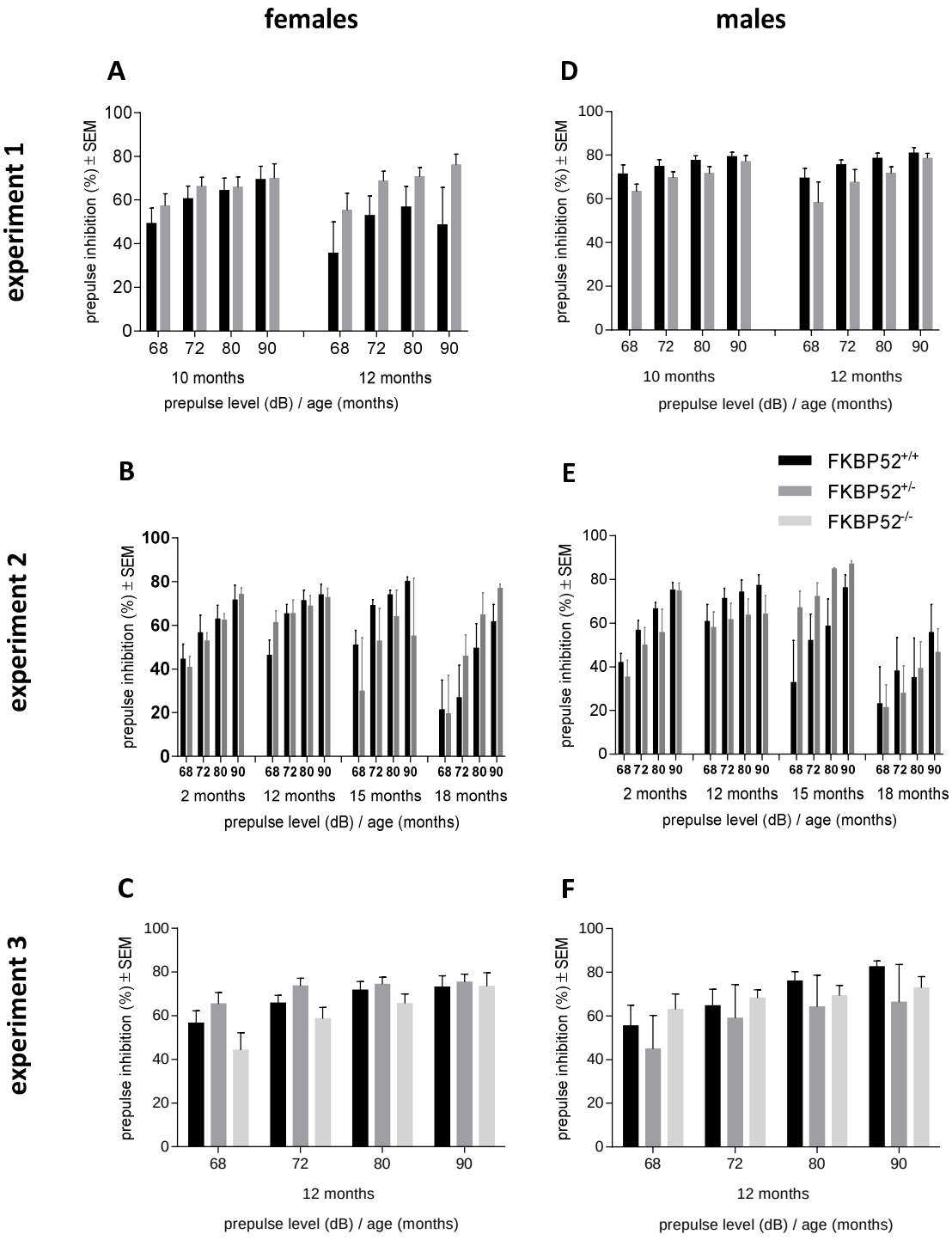
3.5 Acoustic startle magnitude



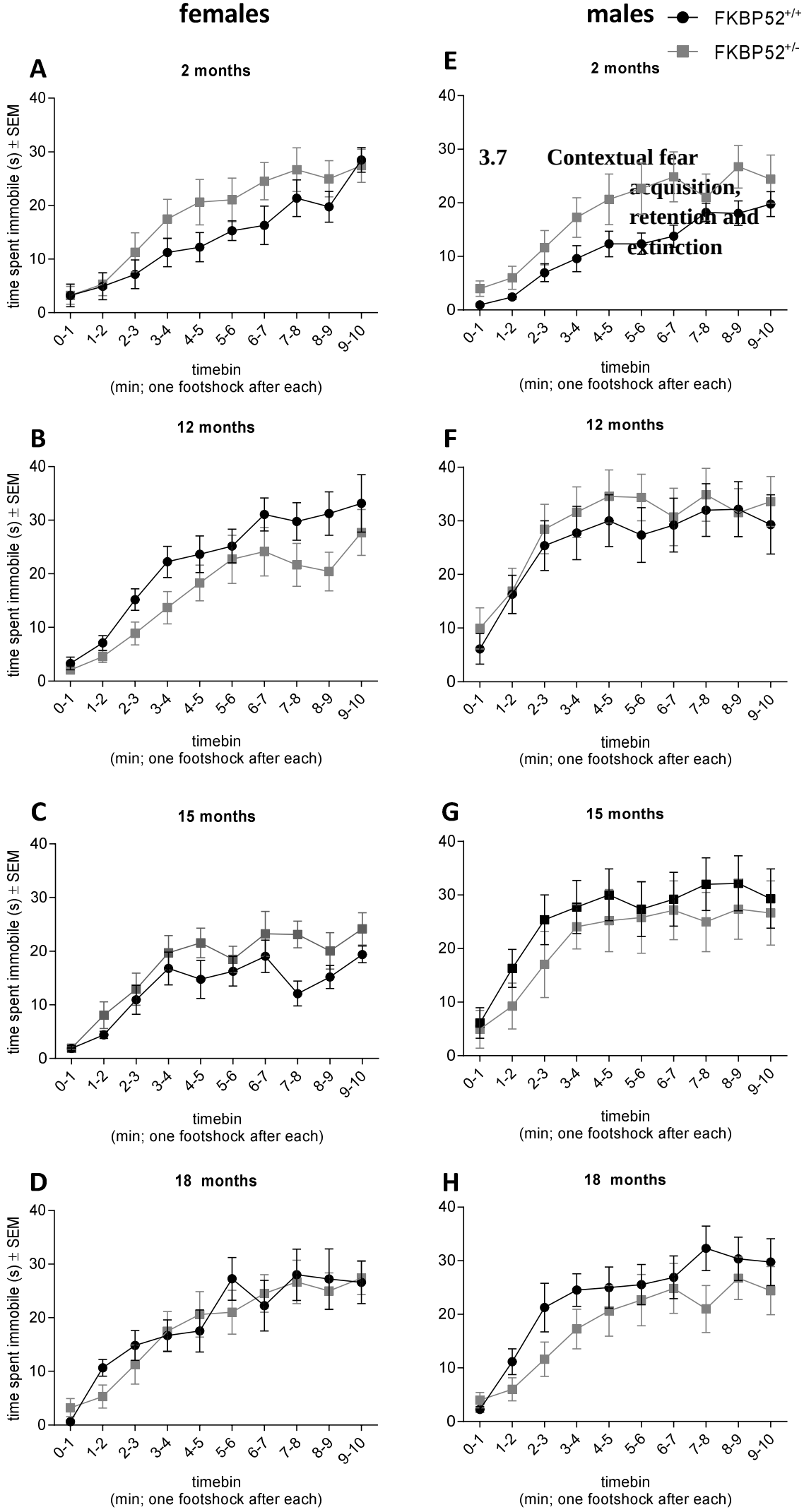
Supplementary Figure S6. Startle magnitude values recorded during the prepulse inhibition protocol in male and female FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice at different ages in

three separate experiments. Startle magnitude decreased with age irrespective of genotype or sex in experiment 1 (age: $F_{(1, 42)} = 6.33$, $p = 0.0158$; **S6 A**) and 2 (age: $F_{(3, 150)} = 6.39$, $p = 0.0004$; **S6 B**). KEY: M male, F female.

3.6 Prepulse inhibition

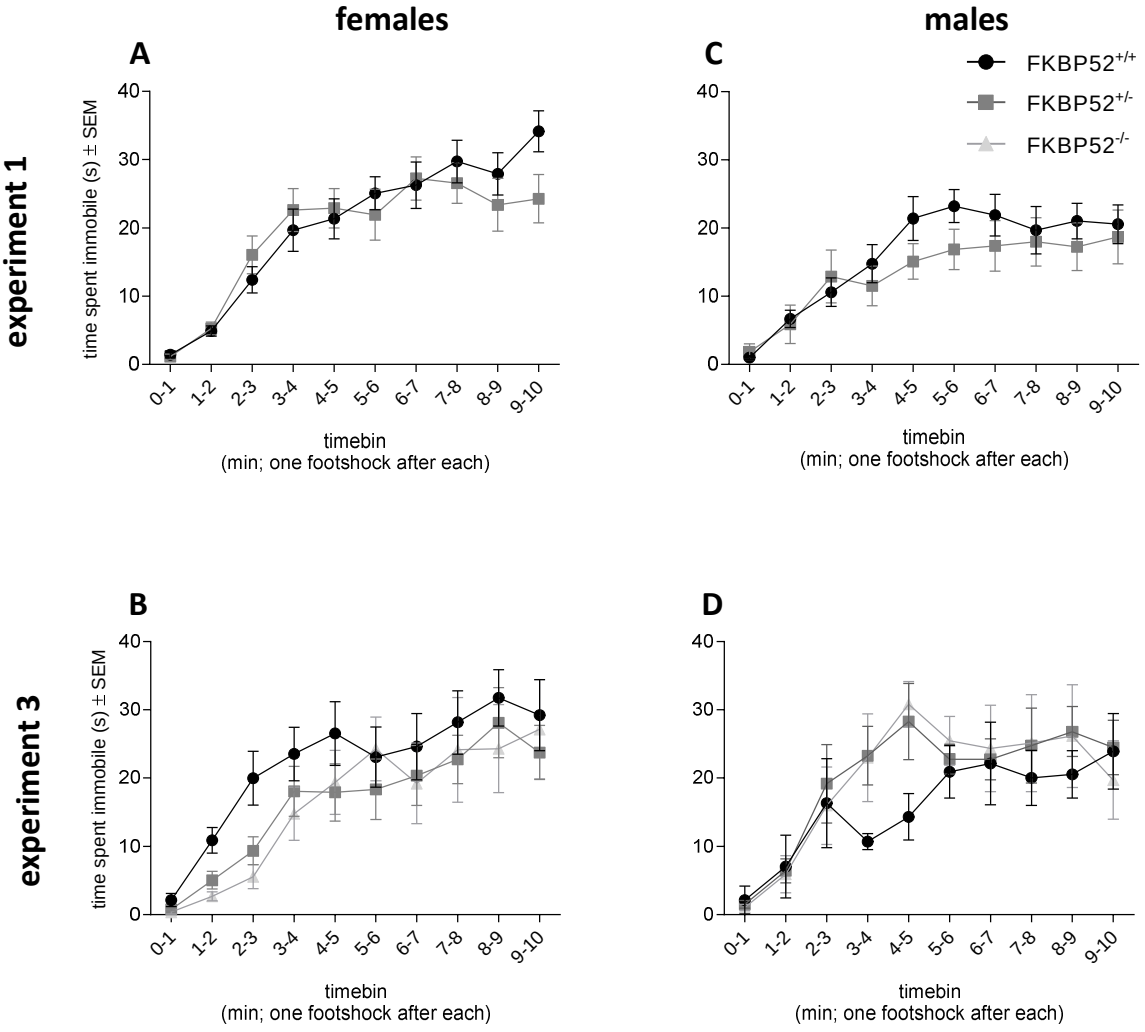


Supplementary Figure S7. dB-level and age-dependent percentage prepulse inhibition in response to prepulse levels of 68, 72, 80 and 90 decibels (dB) by male and female FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice at different ages in experiment 1 **(S7A and D)**, experiment 2 **(S7B and E)** and experiment 3 **(S7 C and F)**. The inhibition of the acoustic startle response increased with the dB levels applied during the prepulse in experiment 1 at ten- (dB level: $F_{(3, 129)} = 20.08$, $p < 0.0010$) and twelve-months-of-age (dB level: $F_{(3, 129)} = 8.71$, $p < 0.0010$; **S7 A**); as in experiment 2 as function of age; whereby inhibition was reduced in eighteen-month-old mice (dB level x age: $F_{(9, 453)} = 4.41$, $p < 0.0010$; **S7 B**).



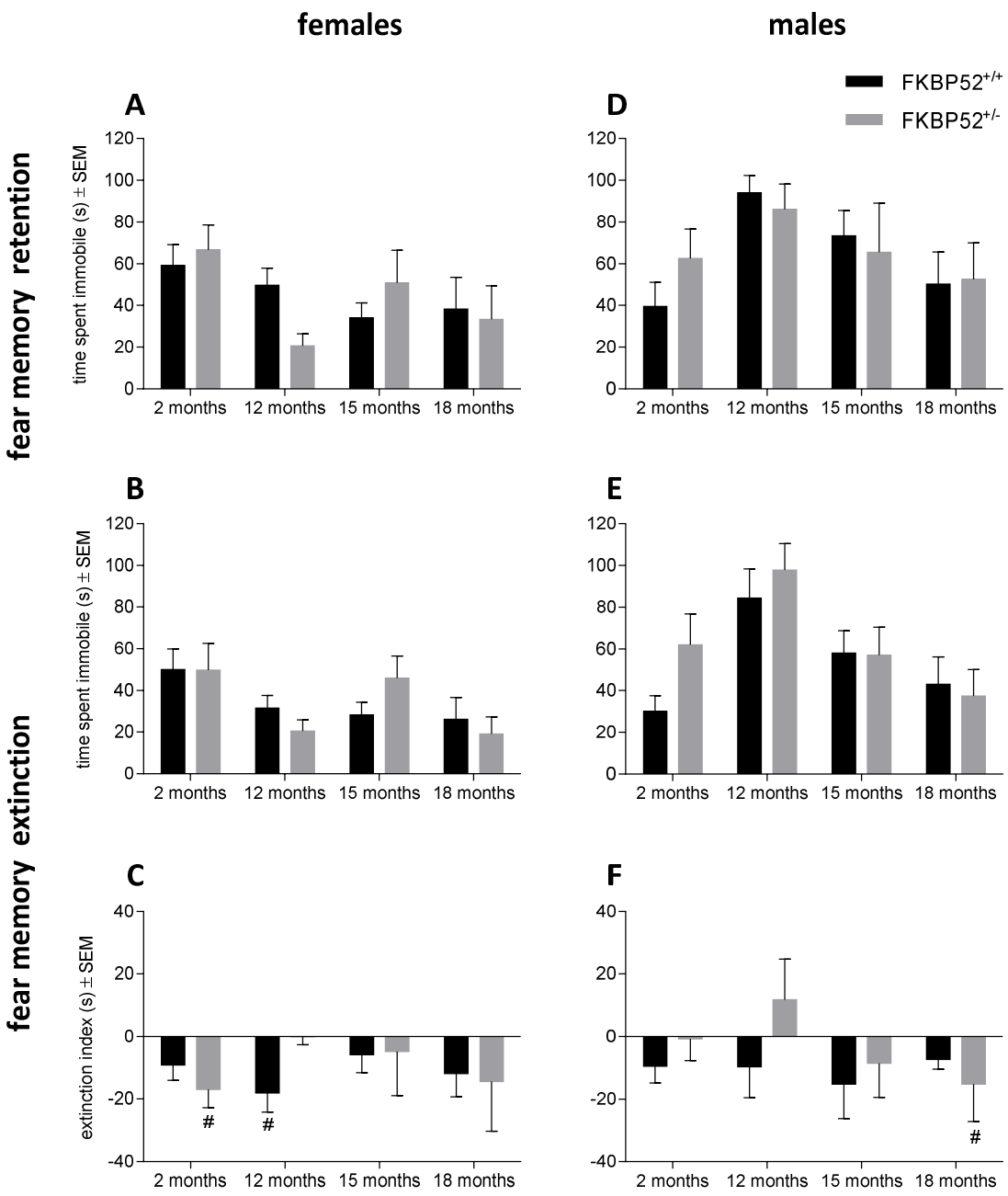
3.7 Contextual fear acquisition, retention and extinction

Supplementary Figure S8. Immobility in response to 0.4 mA foot shocks in the cross-sectional experiment 2 of two- **(S8 A and E)**, twelve- **(S8 B and F)**, fifteen- **(S8 C and G)** and eighteen-month-old **(S8 D and H)** male and female FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice. Both genotypes became increasingly immobile with repeated shock exposure; indicating robust acquisition of contextual fear. The increasing immobility was affected by sex and age (sex x age x shock: $F_{(27, 1341)} = 1.83$, $p = 0.0058$).



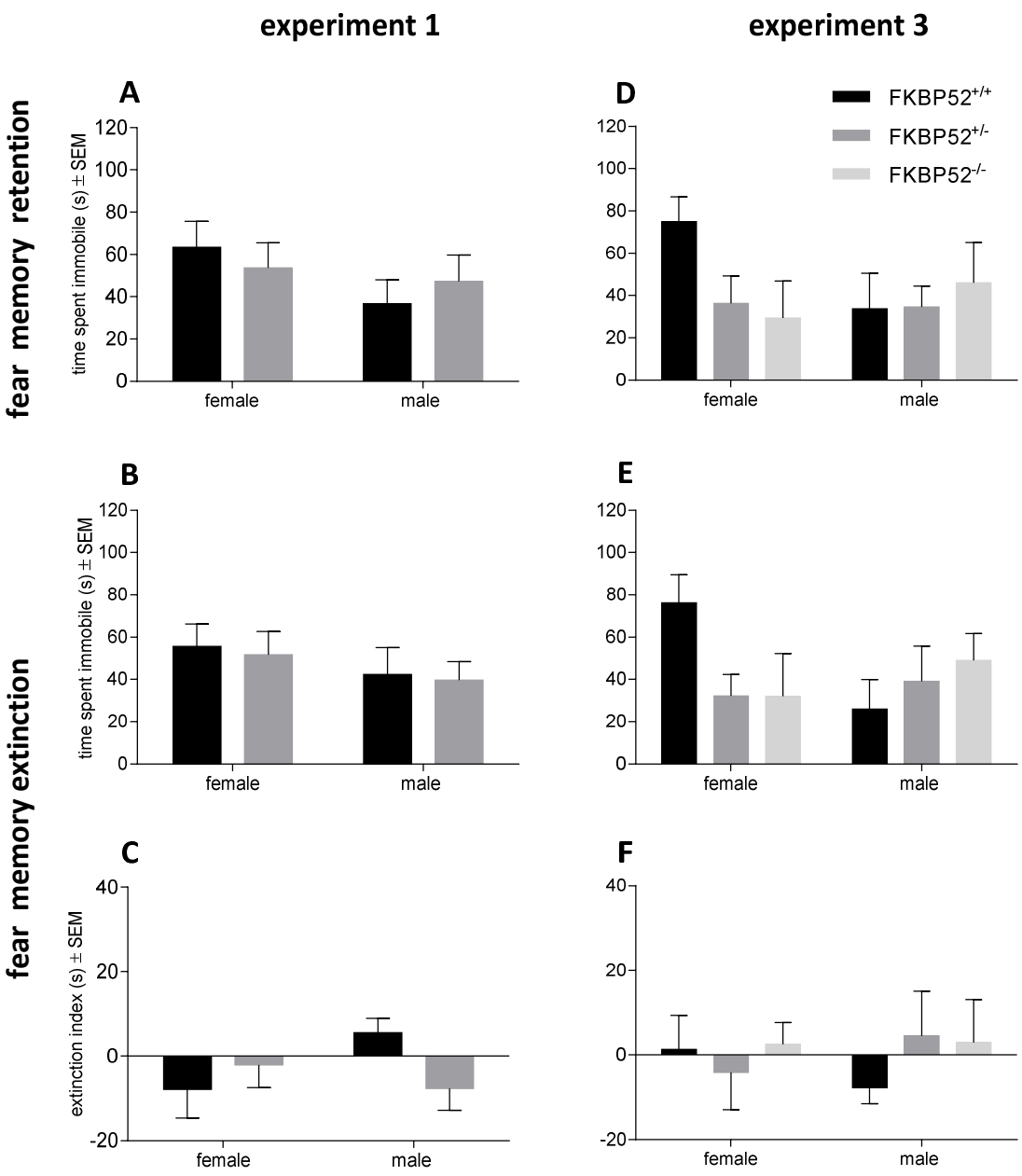
Supplementary Figure S9. Immobility in response to 0.4 mA foot shocks in twelve-month-old mice in experiment 1 **(S9 A and C)** and experiment 3 **(S9 B and D)**

All experimental groups became increasingly immobile with repeated shock exposure, indicating successful acquisition of contextual fear. The increasing immobility was affected by sex in experiment 1 (sex x shock: $F_{(9, 387)} = 3.64$, $p = 0.0002$; **S9 A and C**).



Supplementary Figure S10. Levels of immobility in the retention **(S10 A and D)** and extinction **(S10 B and E)** trials of the experiment 2, following the contextual fear acquisition trial, in male and female FKBP52$^{+/+}$ and FKBP52$^{+/-}$ mice of different ages. Immobility in the contextual fear retention and extinction trials was altered by sex in an age-dependent manner in experiment 2 (sex x age: $F_{(3, 148)} = 5.72$, $p = 0.0010$ and sex x age: $F_{(3, 148)} = 7.65$, $p < 0.0001$, respectively); which was due to twelve-month-old males that remained more immobile than two- ($p = 0.0131$ and $p = 0.0016$, respectively) and eighteen-month-old ($p = 0.0235$ and $p = 0.007$, respectively) males; and which was due to females being less immobile than males in the retention and extinction trial ($p = 0.001$ and $p < 0.001$, respectively).

The extent of contextual fear memory extinction was unaffected by the experimental conditions and no genotype-group showed successful extinction of contextual fear (indicated by negative index values) consistently. KEY: # $p < 0.05$ (one-sample *t*-test, comparison of means to 0).



Supplementary Figure S11. Comparable levels of immobility in retention **(S11 A and D)** and extinction **(S11 B and E)** trials, following the contextual fear acquisition trial, in twelve-month-old female and male FKBP52$^{+/+}$, FKBP52$^{+/-}$ and FKBP52$^{-/-}$ mice from experiment 1 **(S11 A, B and C)** and experiment 3 **(S11 D, E and F)**. Sex-related trends observed in experiment 2 were not apparent in experiment 1 or 3. All groups of experiment 1 **(S11 C)** and 3 **(S11 F)** were unsuccessful in extinguishing contextual fear memory.

4 References

Scullion GA, Kendall DA, Sunter D, Marsden CA, Pardon MC (2009) Central noradrenergic depletion by DSP-4 prevents stress-induced memory impairments in the object recognition task. Neuroscience 164:415-423.