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Sexual dimorphisms in the effect of low-level p25 expression on synaptic plasticity and memory

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Abstract

p25, a degradation product of p35, has been reported to accumulate in the forebrain of patients with Alzheimer's disease. p25 as well as p35 are activators of cyclin-dependent kinase 5 (Cdk5) although p25/Cdk5 and p35/Cdk5 complexes have distinct properties. Several mouse models with high levels of p25 expression exhibit signs of neurodegeneration. On the contrary, we have shown that low levels of p25 expression do not cause neurodegeneration and are even beneficial for particular types of learning and memory [Angelo *et al.*, (2003) *Eur J. Neurosci.*, **18**, 423–431]. Here, we have studied the influence of low-level p25 expression in hippocampal synaptic plasticity and in learning and memory for each sex separately in two different genetic backgrounds (129B6F1 and C57BL/6). Surprisingly, we found that low-level p25 expression had different consequences in male and female mutants. In the two genetic backgrounds LTP induced by a strong stimulation of the Schaffer's collaterals (four trains, 1-s duration, 5-min interval) was severely impaired in male, but not in female, p25 mutants. Furthermore, in the two genetic backgrounds spatial learning in the Morris water maze was faster in female p25 mutants than in male transgenic mice. These results suggest that, in women, the production of p25 in Alzheimer's disease could be a compensation for some early learning and memory deficits.

Introduction

Cdk5 is a small kinase with close structural homology to the mitotic CDKs, which is prominently expressed in postmitotic neurons (reviewed in Dhavan & Tsai, 2001). This kinase is activated by the regulatory subunit p35 (Patrick *et al*., 1998). The p35/Cdk5 complex is required for proper development of the mammalian central nervous system (Beffert *et al*., 2004).

p25, a truncated form of p35, generated by the action of the calcium-activated protease calpain (Kusakawa *et al*., 2000; Lee *et al*., 2000; Nath *et al*., 2000) also binds to Cdk5. However, unlike p35, p25 is not readily degraded, and binding of p25 to Cdk5 constitutively activates Cdk5, changes its cellular location and alters its substrate specificity (Patrick *et al*., 1999; Patzke *et al*., 2003).

Several reports showed that p25 accumulates in the brain of patients with Alzheimer's disease (Patrick *et al*., 1999; Tseng *et al*., 2002; Swatton *et al*., 2004). Alzheimer's disease is characterized by neuronal degeneration, an accumulation of insoluble amyloid peptide in the extracellular space, and neurofibrillary tangles inside neurons. The amyloid peptide derives from the cleavage of the transmembrane amyloid precursor protein (APP). The neurofibrillary tangles are composed of hyperphosphorylated tau, a microtubule-associated protein (Mandelkow & Mandelkow, 1998). Town *et al*. (2002) have shown that amyloid peptide induces an increase in p25 expression and activates the p25/Cdk5 pathway leading to hyperphophorylation of tau.

Several transgenic mice have been generated to study the role of p25 expression. In the majority of these transgenic mice p25 was expressed ubiquitiously at high levels throughout the brain, a situation found to be associated with severe neurodegeneration (Ahlijanian *et al*., 2000; Bian *et al*., 2002; Cruz *et al*., 2003; Noble *et al*., 2003; Shelton & Johnson, 2004). In our model where p25 is expressed in mice at a low level in a forebrain-restricted manner, no signs of neurodegeneration were found (Angelo *et al*., 2003). Furthermore, we showed that p25 expression in the C57BL/6 background improved reversal learning in Morris water maze and altered fear conditioning. These results showed, for the first time, a beneficial effect of an Alzheimer disease-associated process.

The first aim of this study was to assess whether our former findings (Angelo *et al*., 2003) could be replicated in another genetic background, the 129B6F1 background. Discovering some discrepancies, we tested each sex separately and surprisingly, we found a sex-linked difference; in the 129B6F1 and C57BL/6 background p25 expression improved spatial learning only in female mice. Furthermore, late-LTP (L-LTP) at CA1 synapses was significantly reduced in male but not female mice.

Experimental procedures

Animals

Heterozygous p25 transgenic mice (Angelo *et al*., 2003) were obtained in the C57BL/6 background and in the hybrid 129B6F1 (F1, C57BL/6 × 129S2/Sv) background by breeding. C57BL/6 and 129S2/Sv were provided by Harlan UK. The p25 mutants and wild-type (WT) littermates were housed in groups of two to five and treated according to the Animals Act 1986, UK.

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Immunoblot analysis

Whole hippocampus, microdissected hippocampal subregions (CA1, CA3, dentate gyrus), and amygdala were used for immunoblotting. Protein lysate from p25 mutants and wild-type littermates were prepared according to standard methods using a buffer containing phosphatase and protease inhibitors. Proteins were separated on 4–16% polyacrylamide gels and transferred onto PVDF membranes (Bio-Rad, UK). Blots were probed with a primary antibody directed against the C-terminus of p35 (Santa Cruz, 1 : 200) and the signals were visualized with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescent system (Pierce, USA). Signals from the X-ray film were quantified with Densitometer Quantity One (Bio-Rad, UK) in the linear range. Blots were stripped with stripping buffer (Pierce, USA) and reprobed with anti-β-actin to normalize for protein amount.

Morris water maze studies

Naïve male and female mice (8–9-months old) were studied blind to genotype in the hidden-platform version of the Morris water maze. The diameter of the swimming pool was 1.5 m, that of the platform was 10 cm. The water temperature was 22–24 °C. After 10 days of handling, the mice were trained with two trials per day for 10 days. The maximal trial length was 90 s, and the intertrial interval was 60 s. Just before each training trial the mouse was placed on the platform for 60 s. Probe trials were given at day 4, 6, 8 and 10. In these trials the platform was removed. Then the mouse was placed into the swimming pool at the opposite quadrant and allowed to swim for 60 s. At day 11 the platform was moved to the opposite quadrant and the training was continued for 4 days with a probe trial at day 14. This second learning, called reversal learning, tested the ability of the mice to remember a new target site. Video tracking and the HVS 'water 2020' program were used to analyse swimming speed and search strategies. One-way analysis of variance (ANOVA) and Tukey *posthoc* tests were used for statistical analysis.

Fear conditioning

Nine- to ten-month-old mutants and control littermates were tested blind to genotype. Mice used for training had been tested in Morris water maze one month before. Male and female mice were tested 24 h after training for contextual and cued fear conditioning (with a 3-h interval). For the training period of both contextual and cued conditionings each mouse was placed into the conditioning chamber (Campden Instruments, Loughborough, UK) in a soundproof box. After a 120-s period a tone (80 dB, 2.8 kHz) was presented for 30 s, the last 2 s of which coincided with a mild foot shock (0.55 mA). After 30 s the mouse was returned to its home cage. Contextual fear conditioning was tested by re-exposing the mice to the conditioning chamber for 5 min, and cued fear conditioning was tested by exposing the mice to a novel chamber for 3 min without tone presentation, followed by 3 min with tone presentation. The behaviour of the mice was videotaped, and freezing was assessed every 5 s (freezing was scored if no movements other than respiratory were detected within 2 s). One-way ANOVA was used for statistical analysis.

Slice electrophysiology

The animals were decapitated under pentobarbital (80 mg/kg i.p.). The hippocampus was dissected out and cut in 450-μm-thick slices with a tissue chopper. Hippocampal slices were perfused with artificial cerebrospinal fluid (ACSF) with the following composition: 124 mM NaCl, 5 mM KCl, 26 mM $NaHCO_3$, 1.24 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 10 mM glucose, bubbled with a mixture of 95% O_2 and 5% CO_2. Mice were anaesthetized with ether and decapitated. The slices were transferred into the recording chamber and kept in submersion at 28 °C for 1.5 h during which the ACSF was perfused at 2 mL/min. Afterwards, the slices were placed in interface and the perfusion rate reduced to 1 mL/mL. Bipolar twisted nickel-chrome electrodes (50 μm each) were used to stimulate Schaffer's collaterals.

Extracellular field excitatory postsynaptic potentials (fEPSP) were recorded in the stratum radiatum of the CA1 region with low resistance (2–5 megaOhm) glass microelectrodes filled with ACSF. Test stimuli were biphasic (0.1 ms for each pulse) constant-voltage pulses delivered every minute with an intensity adjusted to evoke an approximate 33% maximal response. The slope of the fEPSP was measured on the average of four consecutive responses. For each slice, an input–output curve was established from the responses obtained for various stimulus. Paired-pulse facilitation was tested at four different time intervals: 50 ms, 100 ms, 150 ms and 200 ms. LTP was induced by applying either a single train of stimulation (100 Hz, 1 s) or four trains (100 Hz, 1 s, 5 min interval). In both protocols, the potentiated response was recorded for 4 h.

For each slice, the fEPSP slopes were normalized with respect to the mean slope of the fEPSPs recorded during the 30-min period preceding the induction of LTP. To determine whether or not the normalized fEPSP of a group of slices submitted to the same experimental conditions was significantly potentiated ($P < 0.05$), the percentages of baseline measured just before, 1 h after, and 4 h after induction of LTP were compared by a paired Student's *t*-test. Statistical significance ($P < 0.05$) of the difference in increase of fEPSP measured 1 h and 4 h after the induction of LTP in two distinct groups of slices was assessed by a Student's *t*-test.

In vivo *electrophysiology*

Male wild-type and p25 mutant mice, 8–9-months old, were anaesthetized with urethane (1.8 g/kg i.p.), and held in a semister-eotaxic apparatus. A glass recording pipette, filled with ACSF containing pontamine sky blue was placed 2.1 mm posterior and 1.7 mm lateral to bregma and advanced into the dentate gyrus. A bipolar stimulating electrode (Rhodes SNE 100) was inserted on the same side 3.1 mm lateral to lambda and advanced into the angular bundle to activate fibres of the perforant path. The depths of the two electrodes were adjusted to produce maximal responses in the cell body layer. Constant-current stimuli (60 μs duration, intensity in the range 70–120 μA) were delivered at intervals of 30 s, and intensity adjusted to produce a population spike with an amplitude of 1–3 mV. Test stimuli were delivered at 30-s intervals for 20 min prior to induction of LTP and for 2 h after. LTP was produced by six series of six trains of six stimuli at 400 Hz, 200 ms between trains, 20 s between series. For each animal, synaptic responses were expressed as a percentage change with respect to the mean of the 20 values obtained before the tetanus. Pairs of pulses (interpulse intervals, 10–100 ms) were used to study paired-pulse facilitation at an intensity to evoke a pure fEPSP of approximately 1 mV. Three responses were collected at each intensity and averaged.

Results

p25 Expression in the 129B6F1 Background

Previously, we showed that transgenic mice expressing low levels of p25 in the C57BL/6 background had an interesting behavioural

phenotype including improved reversal learning in the Morris water maze (Angelo *et al*., 2003). Because C57BL/6 mice are known to perform poorly in hippocampus-dependent learning and memory tasks (Owen *et al*., 1997), we introduced the p25 mutation in 129B6F1 hybrid mice, which are known to be better at learning (Fig. 1A). A biochemical analysis was performed to investigate whether the expression level of p25 was similar to that observed in the C57BL/6 background. Using an antibody directed against the C-terminus of p35 that also recognizes p25, we showed that p25 was expressed in the neocortex and the hippocampus but not in the cerebellum of the 129B6F1 mice (Fig. 1B and C). The level of hippocampal expression of p25 was quantified on four animals and was 43% of that of the endogenous p35. Thus, the expression of p25 in the 129B6F1 background (the present study) was similar to that obtained in the C57BL/6 background (33%, Angelo *et al*., 2003). Expression of p25 was the same in male (TG 1 in Fig. 1B and C) and female (TG 2 in Fig. 1B and C) p25 mutants. We can consider this expression as low in comparison with those observed in other transgenic mice where p25 was expressed at least at the same level as p35 (Ahlijanian *et al*., 2000; Bian *et al*., 2002).

We also performed microdissections and found that p25 was expressed in each of the three major areas of the hippocampus (CA1, CA3 and dentate gyrus) as well as in the amygdala (Fig. 1D).

Spatial learning and fear conditioning in p25 transgenic mice

Previously, we studied spatial learning and memory of p25 mutants in the C57BL/6 background in the hidden-platform version of the Morris water maze using a sex-balanced population, without any attempts to distinguish male from female behaviour. In those experiments, p25 mutants had normal initial spatial learning and were found to have improved reversal learning when the platform position was changed after an initial training session of 12 days (Angelo *et al*., 2003). Here we first tested a sex-balanced population of the p25 mutants in the 129B6F1 background in the hidden-platform version of the Morris water maze. The animals were trained for ten days; then the platform position was changed to the opposite quadrant and reversal learning was studied for four more days (Fig. 2A). The latency to reach the hidden platform was not different between WT ($n = 15$; eight females and seven males) and p25 mutant mice ($n = 15$; nine females and six males) either during initial spatial learning or during reversal learning (Fig. 2A) (two-way ANOVAs with repeated measures, $F = 0.831$, $P > 0.05$). However, escape latencies are not such a sensitive measure for spatial learning and memory, because mice can use nonspatial strategies to efficiently locate the hidden platform (e.g. Peters *et al*., 2003). Therefore, probe trials were performed after initial training and at the end of reversal training. During probe trials mutant mice (mixed male and female) swam more slowly than WT mice (0.22 m/s for mutants vs. 0.25 m/s for WT mice, one-way ANOVA, $P < 0.05$), spent longer time in very slow movement (12% for mutants vs. 9% for WT, one-way ANOVA, $P < 0.05$) and spent less time in thigmotaxis (swimming near the pool perimeter; 1.6% for mutants vs. 6% for WT, one-way ANOVA, $P < 0.05$). Despite this, WT and mutant mice searched equally well during the probe trial at the end of the initial training. WT mice searched selectively in the target quadrant where they spent 35% of their time (one-way ANOVA, $F = 10.5$, $P < 0.0001$). Transgenic mice spent 43% of their time in the target quadrant (one-way ANOVA, $F = 50.8$, $P < 0.0001$), a value not statistically different from that of the WT (one-way ANOVA, $F = 2.56$, $P = 0.12$). We also performed a probe trial at the end of the reversal learning and we did not replicate the previous result obtained in the C57BL/6 background (Angelo *et al*., 2003). Both WT and transgenic mice searched selectively in the new target quadrant; both of them spent 35% of their time in this quadrant (one-way ANOVA, $F = 39.1$, $P < 0.0001$ for WT and $F = 19$, $P < 0.001$ for TG). All the animals performed correctly when the platform was visible showing that their swimming ability, motivation and vision were normal.

Our previous study in the C57BL/6 background showed that the p25 mutants had reduced contextual conditioning and enhanced cued

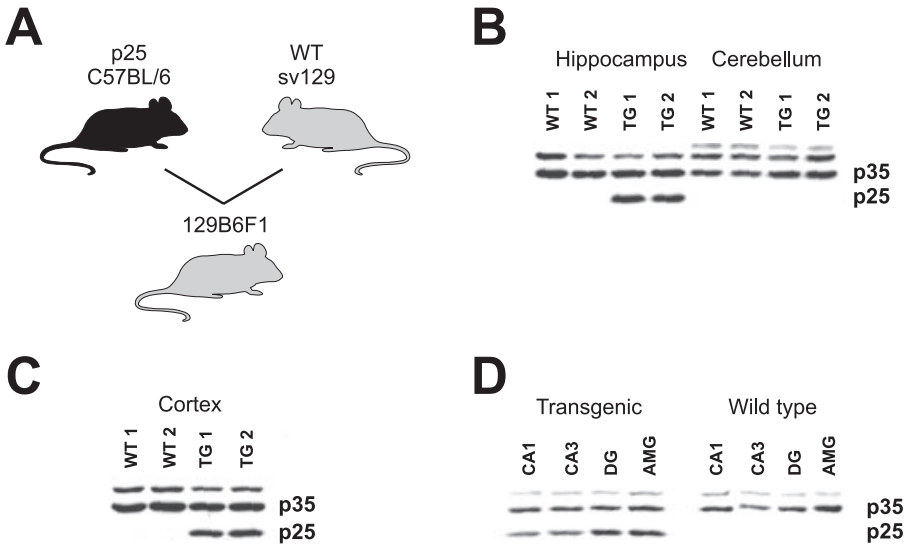

FIG. 1. p25 expression in the 129B6F1 background. (A) Breeding strategy to obtain mice in the 129B6F1 background. (B) p25 and p35 expression revealed by immunoblots using protein from hippocampus, cerebellum in WT or transgenic mice (TG). WT 1 and TG 1 refer to male mice while WT 2 and TG 2 refers to female mice. (C) Expression of p25 and p35 in neocortex in WT and mutant (transgenic) mice. (D) Expression of p25 and p35 in microdissected CA1 region of the hippocampus, CA3 region of the hippocampus, dentate gyrus (DG) and amygdala (AMG) in WT and mutant mice. Notice that p25 expression is restricted to the forebrain where its level of expression is approximately 50% of that of p35.

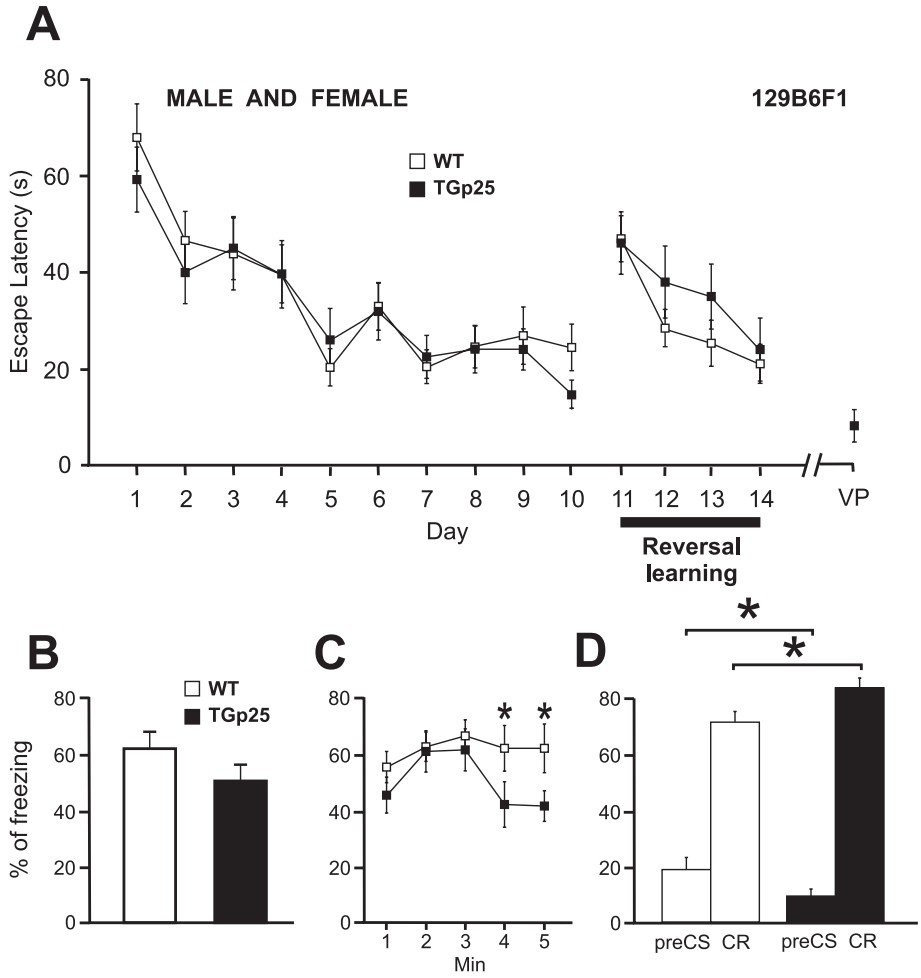

FIG. 2. Spatial learning and memory in a sex-balanced population of p25 mutant mice in the 129B6F1 background. Means ± SEM are shown. ★$P < 0.05$. (A) Acquisition curve during the 10 days of learning and 4 days of reversal learning in the Morris water maze. Visible platform (VP) has been used as control for motivation. No difference in learning and reversal learning was observed between WT (squares) and p25 mutants (filled squares). (B–D) Cued and contextual fear conditioning in p25 mutants in the 129B6F1 background. (B) Contextual freezing measured for five minutes. No difference in contextual fear conditioning was observed between WT mice (white column) and p25 mutants (black column). (C) Contextual freezing measured during each minute. During the last two minutes contextual fear conditioning was reduced in p25 mutants (filled squares). (D) Cued freezing measured for three minutes before tone presentation (preCS) and three minutes during tone presentation (CR). Cued fear conditioning was increased in p25 mutants (black column).

conditioning (Angelo *et al.*, 2003). In the current study, cued and contextual fear conditioning was tested in p25 mutants (six males and nine females) and WT littermates (seven males and eight females) in the 129B6F1 background. Twenty-four hours after training, contextual conditioning was tested first by exposing the animals in the training context for five minutes. When calculated over the whole period, freezing was similar in the two groups (Fig. 2B). However, freezing was reduced in mutant than in WT mice during the last two minutes of the test (Fig. 2C). At that time, p25 mutants froze 40% of the time instead of 60% for the WT (one-way ANOVA, $P < 0.05$). Then, cued conditioning was tested by placing the animals in a new contextual box first for three minutes without tone presentation and afterwards for three minutes with tone presentation. Before tone presentation WT mice froze more than p25 mutants (19% vs. 9%, one-way ANOVA, $F = 5.8$, $P = 0.02$). However, during tone presentation WT mice froze less than p25 mutants (71% vs. 84%, one-way ANOVA, $F = 5.5$, $P = 0.02$) (Fig. 2D). The difference between the percentages of time spent in freezing after and before tone presentation corresponds to tone-induced freezing. It was higher in p25 mutant mice (74%) than in WT mice (53%, one-way ANOVA, $P < 0.001$).

Evidence for a sexual dimorphism in learning and memory in the 129B6F1 background

By screening the data obtained in the 129B6F1 background for potential sex differences, we found that the p25 expression had not the same consequences in male and female mice. In the Morris water maze, female mutants were faster learners than WT mice and male mutants as revealed by a probe trial given after training day 4 (Fig. 3A and B). When the platform was removed at that time, the female mutants searched selectively in the target quadrant (36.6%; one-way ANOVA, $F = 6.43$, $P = 0.0016$), whereas female WT mice (one-way ANOVA, $F = 2.46$, $P = 0.08$) as well as WT and mutant male mice searched randomly ($F = 2.46$, $P = 0.08$ and $F = 2.57$, $P = 0.12$, respectively) (Fig. 3A and B). The improvement observed in female mutant is characterized by a more rapid acquisition of the spatial memory, not by a higher level of selectivity. During probe trial performed at day 6 and 8, mutant female mice showed no improvement in selectivity (37% of time spent in the target quadrant at day 8, one-way ANOVA, $F = 6.5$, $P = 0.0015$) although female WT started to search selectively at day 6 (33.6% of time spent in the target

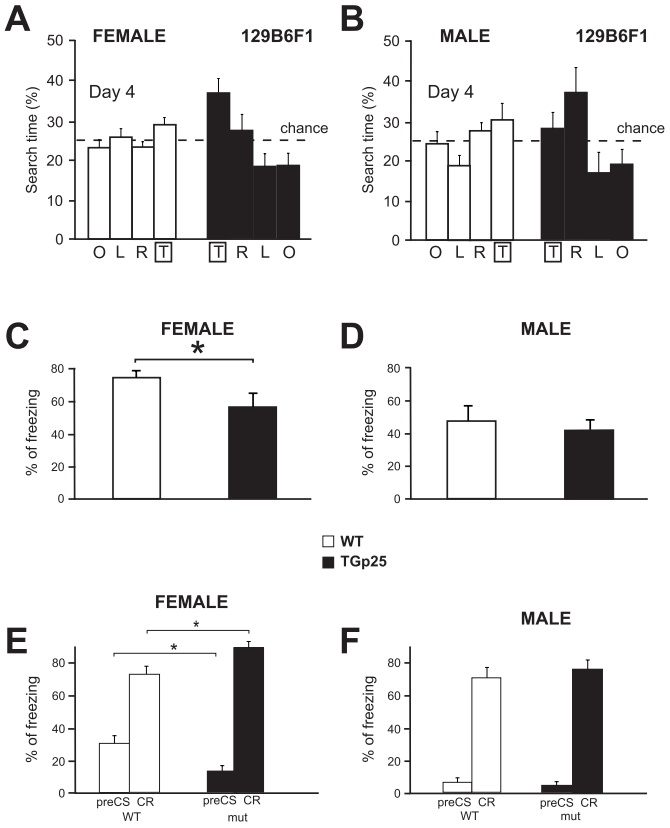

FIG. 3. Evidence for a sexual dimorphism in learning and memory in p25 mutant mice in the 129B6F1 background. Throughout the figure white columns refer to WT mice, black columns to mutant mice. Means ± SEM are shown. (A–B) Morris water maze. Probe trial at day 4 in female (A) and male (B). One-way ANOVA test showed that there was a significant effect of genotype for reaching the initial location of the platform in female mice (p25 mutants were faster learners) but not in male mice. T, target quadrant; O, opposite quadrant; L, adjacent left; R, adjacent right. (C–D) Contextual fear conditioning in female (C) and male (D) mutants and control littermates. p25 expression caused a reduced contextual fear conditioning in female but not in male mice. (E–F) Cued freezing measured for three minutes before tone presentation (preCS) and three minutes during tone presentation (CR) in female (E) and male (F) WT and mutants. p25 expression increased cued fear conditioning in female but not in male mice.

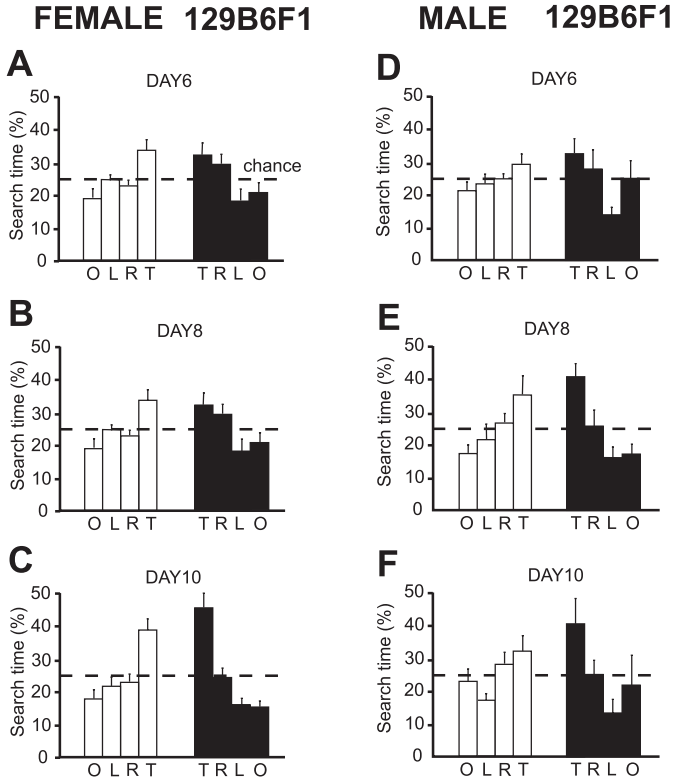

FIG. 4. Morris water maze studies in the 129B6F1 background. Probe trial at day 6, 8 and 10 in female (A–C) and male (D–F) mutants and control littermates. White columns for WT and black columns for mutants. T, target quadrant; O, opposite quadrant; L, adjacent left; R, adjacent right. Female WT mice reached the same level of selectivity than female mutant mice at day 6. Male WT and mutant mice searched selectively in the target quadrant at day 8 and 10.

quadrant, one-way ANOVA, $F = 4.6$, $P = 0.01$). There was no difference between WT and mutant female mice at day 6, 8 or 10 (one-way ANOVA, $P = 0.7$, $P = 0.45$, $P = 0.6$, respectively) (Fig. 4A–C). Male WT and mutant mice showed an identical spatial memory becoming selective at day 8 (35.3% vs. 40.9% of time spent in the target quadrant, one-way ANOVA, $F = 4.3$, $O = 0.01$ and $F = 8.8$, $P = 0.0006$, for WT and mutant, respectively) (Fig. 4D–F).

This sexual dimorphism was also observed in fear conditioning. During the test of contextual conditioning, female p25 mutant mice froze significantly less (56%) than female WT (76%) (one-way ANOVA, $F = 4.84$, $P = 0.044$). (Fig. 3C). By contrast, no differences were observed between mutant (42%) and WT male mice (47%) (one-way ANOVA, $F = 0.24$, $P = 0.63$) (Fig. 3D). During the test of cued conditioning, female mutant froze less than WT before tone (13% vs. 30%, one-way ANOVA, $F = 8.72$, $P = 0.01$) but froze more than WT during tone presentation (89% vs. 73%, ANOVA on ranks, $P = 0.02$) (Fig. 3E). Thus, tone induced more freezing in female p25 mutants (76%) than in WT mice (42%, one-way ANOVA, $F = 85.5$, $P < 0.001$). By contrast, male p25 and WT mice froze equally before the tone (3.7 vs. 5.5%, ANOVA on ranks, $P = 0.63$) and during tone presentation (75.5 vs. 70, one-way ANOVA, $F = 0.42$, $P = 0.53$) (Fig. 3F).

Thus, in the 129B6F1 background low-level expression of p25 improved certain types of memory, not all, and that only in female mice.

Learning and memory in the C57BL/6 background

The finding that low p25 expression affected learning and memory in a sex-specific way in the 129B6F1 background, urged us to re-analyse data for sex differences in memory tests obtained in our previous work in the C57BL/6 background (Angelo *et al.*, 2003). Re-examining the Morris water maze data revealed that female p25 mutants were faster learners than female WT mice (Fig. 5A). During a probe trial given at day 6, female p25 mutants ($n = 6$) spent 46% of their time in the quadrant where the platform was during training (the target quadrant), which was significantly more than for female WT mice ($n = 8$; 30%; one-way ANOVA, $F = 7.32$, $P < 0.05$). By contrast, male p25 mutants ($n = 8$; 33%) and male WT mice ($n = 6$; 36%) equally searched in the target quadrant (one-way ANOVA, $F = 0.098$, $p = 0.76$) (Fig. 5B). Thus, in the C57BL/6 background female but not male p25 mutants had improved spatial learning.

By contrast, there was no sexual dimorphism in fear conditioning. During the test of contextual conditioning female p25 mutants froze less (46%) than WT mice (71%, one-way ANOVA, $F = 6.46$,

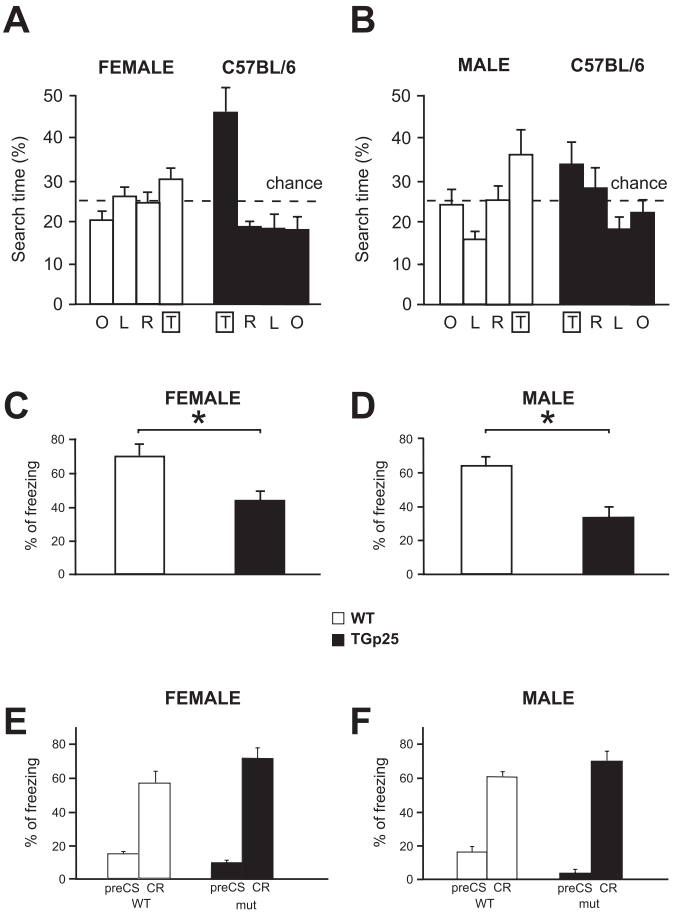

FIG. 5. Evidence for a sexual dimorphism in learning and memory in p25 mutant mice in C57BL/6 background. Symbols and presentation is exactly the same as those used in Fig. 3. (A–B) Morris water maze. One-way ANOVA showed that there was a significant effect of genotype in female mice (p25 mutants were faster learners) but not in male mice. (C–D) Contextual fear conditioning was decreased by p25 expression both in male and female mice. (E–F) Cued freezing measured for three minutes before tone presentation (preCS) and three minutes during tone presentation (CR). Cued fear conditioning was increased by p25 expression both in male and female mice.

$P < 0.05$) as did male p25 mutants (35% for mutants vs. 66% for WT mice; one-way ANOVA, $F = 11.3$, $P < 0.01$) (Fig. 5C and D). During the test of cued conditioning, the proportion of time spent freezing, which was caused by the tone presentation, was higher both in female p25 mutants compared to female WT mice (63% and 43%, respectively, one-way ANOVA, $F = 6.68$, $P < 0.05$) and in male p25 mutants compared to male WT mice (68% and 45%, respectively, one-way ANOVA, $F = 7.66$, $P < 0.05$). Female mutant mice froze less than WT before tone (10.2% vs. 15,7%) but more during tone presentation (73.6% vs. 58.3%). In the same way, male mutant mice froze less than WT before the tone (3.7% vs. 16.7%) but more during tone presentation (71.8% vs. 62.5%) (Fig. 5E and F).

LTP in CA1 synapses in male and female p25 transgenic mice in the 129B6F1 background

Considering that female and male p25 mutants in the 129B6F1 background did not perform equally in hippocampus-dependent learning and memory tests, the influence of low level p25 expression on hippocampal LTP was also analysed for each sex separately.

In female WT mice ($n = 7$), four tetani induced an increase in fEPSP slope to 239%, which was maintained at 213% four hours later (a value statistically different from the baseline, paired Student's *t*-test, $P < 0.05$). Figure 6A shows that this potentiation was not different from that obtained in female mutant mice ($n = 7$) where the potentiation was maintained at 196% four hours after the trains (Student's *t*-test, $P > 0.05$). However, a significant impairment in the LTP induced by four tetani was observed in male p25 mutants (Fig. 6B). In male WT mice ($n = 6$) four tetani induced a potentiation of the response to 281% of the basal level and this potentiation was maintained for four hours (247% at the fourth hour). In male mutants ($n = 6$) four tetani induced a smaller immediate potentiation (228%), which was maintained for four hours at a lower level than in WT mice (164% at the fourth hour). This reduction was statistically significant (Student's *t*-test, $P < 0.05$).

Figure 6C shows that the LTP induced by a single tetanus was larger in the female p25 mutants ($n = 5$) than in WT mice ($n = 5$). Immediately after the tetanus the potentiation was similar in both groups (206% in WT vs. 216% in p25 mutants, Student's *t*-test $P > 0.05$). Thereafter, the potentiation decreased slowly in WT mice to 156% and to 162% two hours and four hours after the train, respectively, whereas in p25 mutant it was maintained at 192% and 205% two hours and four hours after the stimulation (Student *t*-test, $P < 0.05$) (Fig. 6C). On the contrary, LTP induced by one tetanus was reduced in the male p25 mutants although this was not statistically significant (Fig. 6D). In WT mice ($n = 4$) the potentiation reached 241% immediately after the trains and was still at 191% four hours later. In male mutants ($n = 4$) the initial potentiation was slightly reduced (217%). Afterwards the potentiation was maintained at 162% four hours after the tetani (a level not statistically different from that of the controls, Student's *t*-test, $P > 0.05$).

The observed LTP deficit in male p25 mutants was not accompanied by defects either in basal synaptic transmission or in paired-pulse facilitation (PPF), a classical measurement of presynaptic short-term plasticity (Fig. 6E and H). In male, the input/output curves were similar in WT ($n = 8$) and p25 mutant mice ($n = 9$) whereas a small enhancement of PPF in mutant mice was observed when the interval between the two pulses was 100, 150 or 200 ms (Student's *t*-test, $P < 0.05$) (Fig. 6F and H). In female p25 transgenic mice ($n = 7$) basal synaptic transmission and PPF were similar to those observed in female WT mice ($n = 7$) (Fig. 6E and G).

LTP in CA1 synapses in male and female p25 transgenic mice in the C57BL/6 background

The observation that low p25 expression in the 129B6F1 background caused a defect in the LTP induced by four trains, specifically in male mice, led us to check whether this finding was related to the genetic background. We thus studied the LTP induced by four trains on slice from transgenic p25 and wild-type C57BL/6 mice, separating male and female mice.

In the C57BL/6 background, LTP induced by four tetani in female transgenic mice was normal (Fig. 7A). The fEPSP slope was potentiated at 270% in WT and 254% in transgenic mice immediately after the tetani. Thereafter, the potentiation was maintained four hours after the tetani at 212% in WT and at 219% in transgenic mice (Student's *t*-test $P > 0.05$). However, as in the 129B6F1 background, male C57BL/6 transgenic mice showed a severe impairment in the LTP induced by four tetani (Fig. 7B). Four tetani induced an immediate potentiation similar in WT

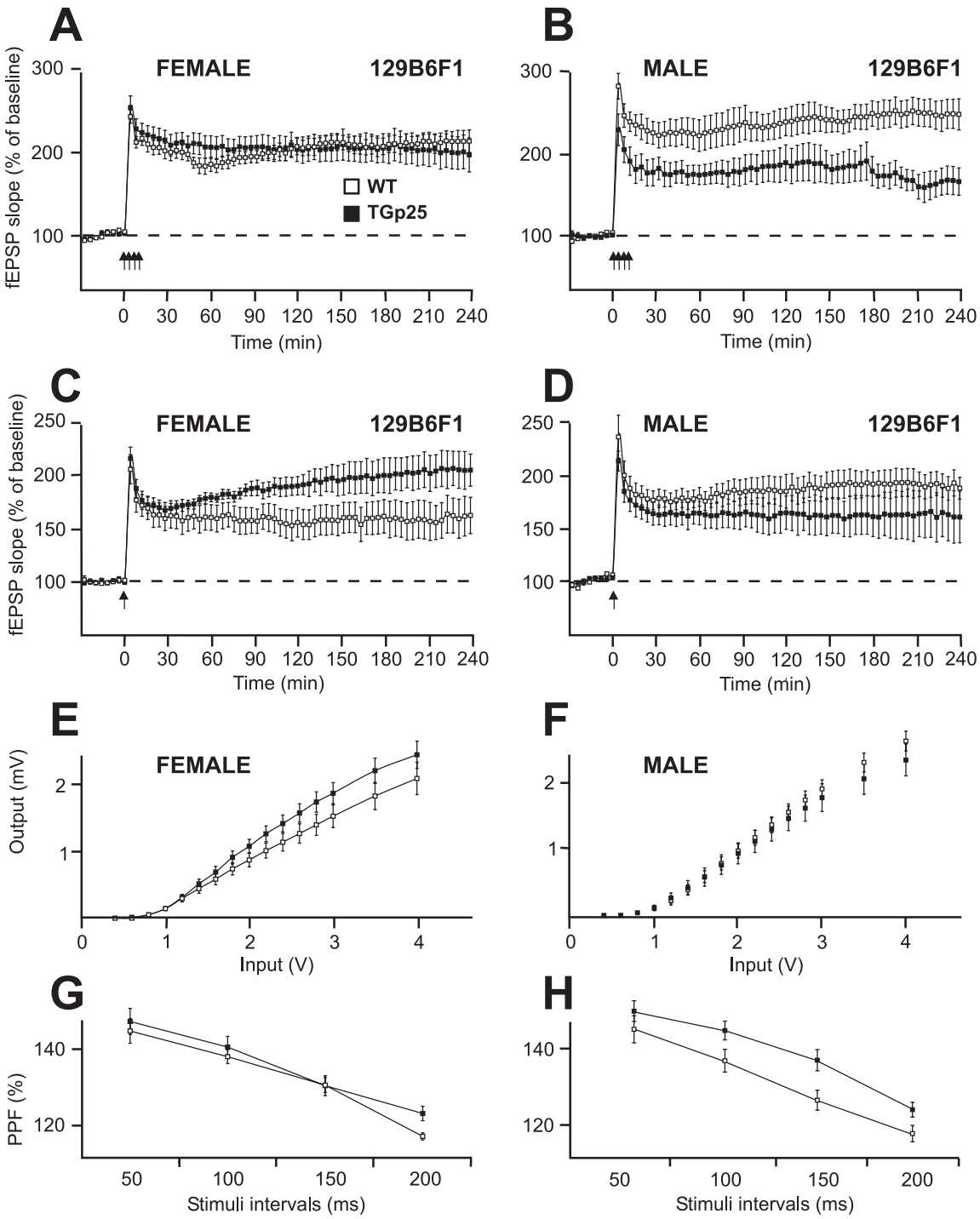

FIG. 6. In the 129B6F1 background LTP is normal or increased in female p25 mutant mice whereas it is reduced in male p25. Means ± SEM are shown. Mutants, filled squares; WT mice, squares. (A) LTP induced by four tetani (100 Hz, 1 s, 5 min interval) in female mice. (B) LTP induced by four tetani (100 Hz, 1 s, 5 min interval) in male mice. (C) LTP induced by one tetanus (100 Hz, 1 s) in female mice. (D). LTP induced by one tetanus (100 Hz, 1 s) in male mice. (E–H) Basal synaptic transmission and paired-pulse facilitation (PPF) in p25 mutant mice in the 129B6F1 background. (E–F) Input/output curve in female mice (E) and in male mice (F). (G–H) PPF in female mice (G) and in male mice (H).

(270%, $n = 4$) and mutant mice (272%, $n = 4$, Student's t-test, $P > 0.05$). This potentiation remained sustained in WT mice (284% after 4 h), whereas it was reduced to 184% after 1 h and to 132% after 4 h in the male mutants. These values were significantly different from the baseline (paired Student's t-test, $P < 0.05$) and they differed significantly from the potentiation values obtained in WT mice (Student's t-test, $P < 0.05$).

In vivo *LTP in dentate gyrus in male p25 mutants in the C57BL/6 background*

We also checked whether low-level expression of p25 in male mice in the C57BL/6 background affected another type of LTP that was induced in the dentate gyrus of anaesthetized animals *in vivo* by repetitive stimulation of the perforant pathway. We found no

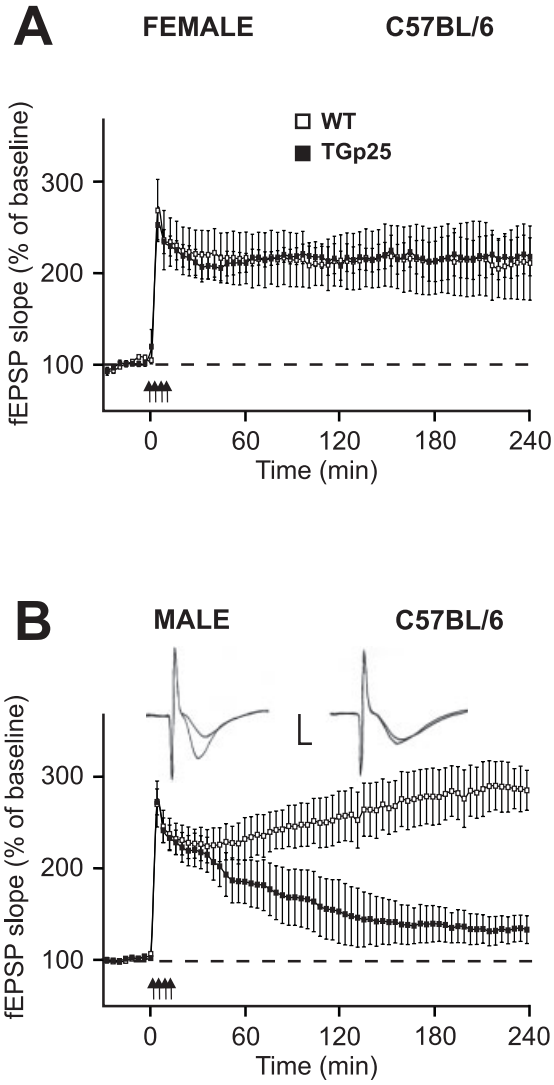

FIG. 7. In the C57BL/6 background, LTP induced by four tetani was severely impaired in male but not in female p25 mutants. Recordings were performed in the CA1 region. Stimulation was applied to the Schaffer's collaterals. (A–B) LTP was induced by four tetani (100 Hz, 1 s, 5 min interval) in female mice (A) and in male mice (B) separately.

significant differences between p25 mutants and WT mice (Fig. 8A–C). The fEPSP slope at 120-min post-tetanus was increased by 5.1 ± 2.7% ($n = 4$) in mutants and by 8.3 ± 4.1% ($n = 4$) in WT mice. Similarly, there was no significant difference in the spike potentiation, which was 336.7 ± 96% in mutants compared to 424.3 ± 138% in WT mice ($n = 4$ for both groups). PPF was also similar in the two groups of mice (Fig. 8D). The shown traces are examples of fEPSP recordings just before and 3 h after tetani for WT (left) and mutants (right). Calibration bars, 2 ms and 2 mV.

Discussion

Summary

We have found that low-level p25 expression induces sex-dependent modifications in long-term potentiation and in behavioural memory tests. In male, but not in female mutants, low-level p25 expression causes a severe defect in the LTP induced by four tetani in the CA1 synapses. Interestingly, this male-restricted defect,

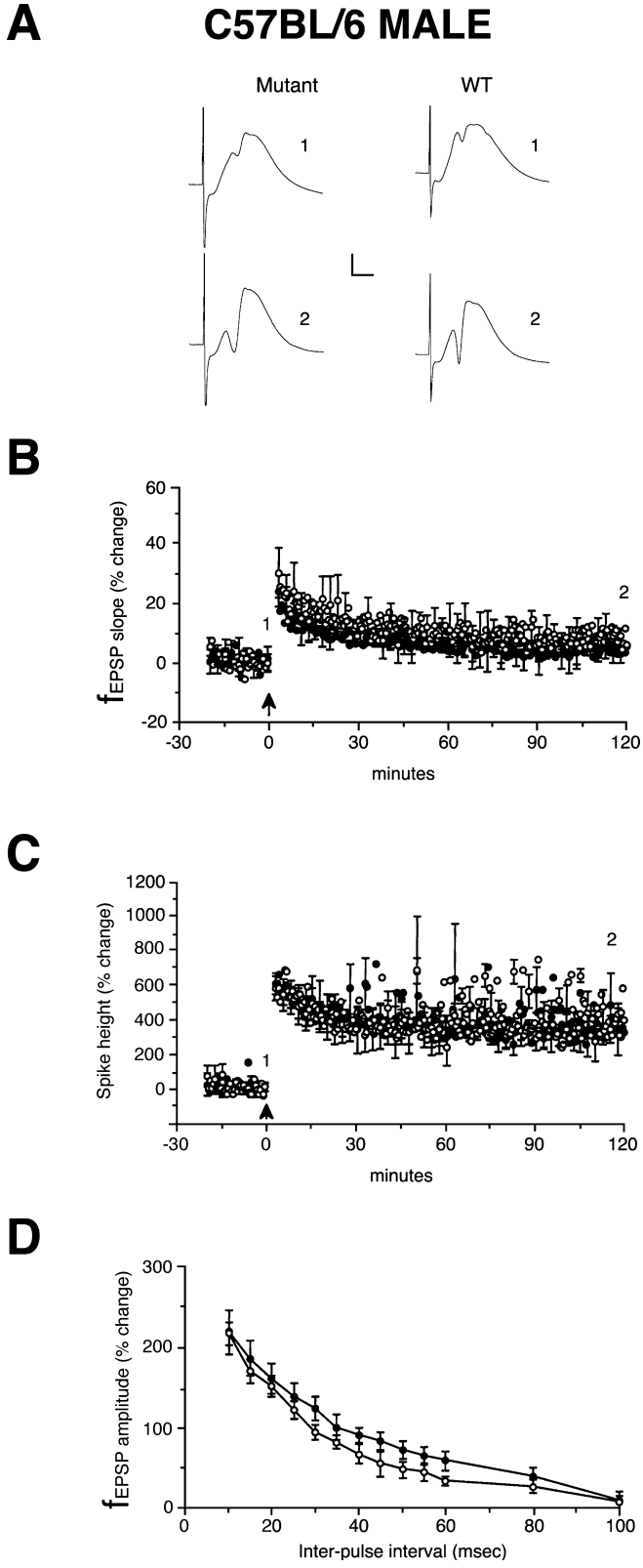

FIG. 8. LTP is normal in the dentate gyrus *in vivo* in male p25 mutants in the C57BL/6 background. Means ± SEM are shown. Mutants, filled circles; WT mice, open circles. (A) Representative recordings directly before (1) and 120 min after the tetanus (2). Calibration bars, 3 ms and 4 mV. (B–C) Normal dentate gyrus LTP in the p25 mutants. The arrow indicated the tetanus. (B) fEPSP slope. (C) Amplitude of the population spike. (D) PPF is indistinguishable between WT and p25 mutants.

which is present both in the 129B6F1 and in the C57BL/6 genetic backgrounds, is accompanied with no impairments either in spatial learning and memory or in cued fear conditioning, or in contextual fear conditioning. In female, but not in male mutants, low-level p25 expression induces a faster learning in the Morris water maze also, in the 129B6F1 and in the C57BL/6 genetic backgrounds. Female restricted modifications in fear conditioning is also present but only when p25 is expressed in the 129B6F1 genetic background. In female p25 mutants in that genetic background cued conditioning is increased while contextual conditioning is reduced.

Penetrant phenotypes caused by p25 expression in different genetic backgrounds

Genetic and phenotypic variations between inbred mouse strains that are used to construct genetic models may confound the interpretation of cellular neurophysiological and behavioural data observed in these models. Several studies have demonstrated that there are strain-dependent differences in LTP and learning and memory (Nguyen *et al*., 2000; Waddell *et al*., 2004). Consequently, the phenotype of a mutation can vary with the genetic background. However, when the phenotype persists in different genetic backgrounds, the full impact of the mutation is ascertained. Here we have studied the effects of low-level p25 expression on learning and memory and synaptic plasticity in two different genetic backgrounds (C57BL/6 and 129B6F1). Only the results obtained in the two backgrounds can be indisputably related to p25 expression. Results obtained in one genetic background and not in the other must be seen more as a small contribution influenced by modifiers specific for a particular genetic background. For example, we previously found that p25 expression caused improved reversal learning in the Morris water maze in the C57BL/6 background (Angelo *et al*., 2003). Here we found that the same genetic modification introduced in the 129B6F1 background did not improve reversal learning in the water maze. Thus, the effect of p25 expression on reversal learning is to be considered as small.

However, p25 expression caused a sexual dimorphism (CA1-LTP impairment in male mice and faster spatial learning in the Morris water maze as well as altered fear conditioning in female mice) in both genetic backgrounds. These changes must thus be considered as strongly related to the change in p25 expression.

p25 expression has sexually dimorphic effects on synaptic plasticity and memory

Unexpectedly we found that a sex-specific influence of low level of p25 expression on memory and LTP in mice. So far only a few molecules are known to affect synaptic plasticity and memory in a sex-specific manner. Sex hormones such as oestrogen and testosterone regulate hippocampal memory formation (Daniel & Lee 2004; Korol, 2004). The effects of oestrogen have most intensively been studied. *In vivo*, LTP in the CA1 region of the hippocampus is augmented during the pro-oestrous cycle stage of the oestrous cycle (Good *et al*., 1999). When applied on hippocampal primary neuronal culture, 17-β estradiol induces phosphorylation of CREB via a process mediated by both Ca^{2+}/calmodulin kinase II and mitogen-activated protein (MAP) kinase (Sawai *et al*., 2002; Lee *et al*., 2004). In hippocampal cell cultures, oestrogen stimulates tyrosine phosphorylation of NMDA receptors via the src tyrosine kinase/MAP kinase pathway and enhances LTP (Bi *et al*., 2000). Moreover, in intact animals, oestrogen increase spatial memory tasks (Gibbs, 2000; Rissman *et al*., 2002) whereas oestrogen replacement therapy in women is associated with reduced incidence of Alzheimer's disease (Sherwin, 2003). In addition to the sex hormones the Ca^{2+}/calmodulin kinase kinases have a sex-specific role; they are required for hippocampal memory formation in males but not in females (Mizuno *et al*., 2004).

Here we show that low-level p25 expression changes hippocampal memory formation in female but not in male mice. The p25 expression improved spatial memory formation in the water maze and altered fear conditioning in the 129B6F1 background. In that background contextual conditioning (a tone was presented during conditioning) was reduced but tone conditioning was enhanced. This phenotype in fear conditioning is rarely observed and has been ascribed to enhanced cholinergic function (Angelo *et al*., 2003). Indeed Cdk5 in cholinergic neurons contributes to hippocampal learning (Fischer *et al*., 2002, 2003) and enhanced cholinergic function results in faster spatial learning (Everitt & Robbins, 1997). More investigations are needed to establish whether cholinergic function in memory formation is sexually dimorphic, as suggested by our studies.

Cdk5 and synaptic plasticity

p25 expression overactivates Cdk5 and we found that this alteration of Cdk5 activity impacts on LTP at hippocampal CA1 synapses in male mice. Several substrates of Cdk5 are known to play a role in synaptic plasticity. Presynaptically, Cdk5 phosphorylates P/Q voltage-gated calcium channel (VDCC) (Tomizawa *et al*., 2002) and two proteins implicated in vesicle recycling, dynamin I and amphiphysin I (Tan *et al*., 2003; Tomizawa *et al*., 2003). Postsynaptically, Cdk5 phosphorylates the *N*-methyl-D-aspartate (NMDA) receptor subunit NR2A (Li *et al*., 2001; Wang *et al*., 2003) and the postsynaptic density protein PSD-95 (Morabito *et al*., 2004). Cdk5 also interferes with signalling cascades involving mitogen-activated protein (MAP) kinase, which are known to play a role in synaptic modulation. p42-p44 MAP kinase is a central component of a signal transduction pathway stimulated in LTP (Rosenblum *et al*., 2002; Kelleher *et al*., 2004). Phosphorylation of MEK1 by Cdk5 results in an inhibition of MEK1 catalytic activity and consequently in a reduced phosphorylation of p42-p44 MAP kinase (Sharma *et al*., 2002). Cdk5 can also interfere with a cascade involving p38 MAP kinase. Indeed the block of LTP caused by the amyloid β-peptide is rescued by inhibitors of Cdk5 or of p38 MAPK but not by inhibitors of p42-p44 MAPK (Wang *et al*., 2004).

We observed that low level p25 expression impairs transcription-dependent LTP in male mice. This impairment is explained best by an increased Cdk5 mediated inhibition of MAP kinase activity that is needed for the activation of CREB-dependent transcription (Lonze & Ginty, 2002). It is possible that the female mutants do not have the impairment in transcription-dependent CA1-LTP, because oestrogen may compensate by activating transcription via MAP kinases (Bi *et al*., 2000).

Relationship between LTP and memory formation

LTP has been suggested to underlie memory formation (Bliss & Collingridge, 1993). However, one must be aware that, even in '*in vivo*' LTP, recordings are made from specific cell populations in response to stimulation of a specific set of fibers. In contrast, to this relatively well-controlled situation, memory formation involves different neuronal networks. Moreover, there are multiple facets in memory and multiple forms of LTP (reviewed in Lynch, 2004). In this study male p25 mutants presented an impairment in CA1-LTP but not in LTP induced in the dentate gyrus '*in vivo*'. Moreover, the severe impairment in CA1-LTP observed in male p25 mutants was not

associated with a deficit in spatial and contextual memory formation. Such dissociations between CA1-LTP and memory formation have already been previously reported. CaMKKβ null mutants have impaired late-LTP and are only delayed in spatial memory formation in the Morris water maze (Peters *et al*., 2003). Fmr2 knock-out mice, which show enhanced LTP, are impaired in contextual memory formation (Gu *et al*., 2002). Heterozygous BDNF null mutants have reduced LTP but normal spatial learning in the Morris water maze (Montkowski & Holsboer, 1997). These examples, as well as the dissociation found in this study, suggest that although CA1-LTP is a good model to understand the basic mechanisms through which the strength of a synapse can be changed by activity, it is not a direct measure of hippocampus-dependent memory formation, most likely because distinct plasticities at different synapses are needed in a network system to form a memory.

p25 formation could be a compensation for early learning and memory deficits in female patients with Alzheimer's disease

In our previous study we have suggested that p25 formation could be a compensation for early learning and memory deficits in Alzheimer's disease, because our p25 transgenic mice had improved reversal learning in the Morris water maze (Angelo *et al*., 2003). We now show that improved reversal learning depends on the genetic background and that p25 expression enhances spatial learning in the Morris water maze in female but not male mice. Thus, p25 expression might be a compensation for early learning and memory deficits in female patients with Alzheimer's disease. An up-regulation of the cholinergic system has been suggested to be an early compensatory response in Alzheimer's disease (DeKosky *et al*., 2002). Enhanced cholinergic function could be responsible for the enhanced spatial learning and the altered fear conditioning in the female p25 mutants (Everitt & Robbins, 1997; Chang & Gold, 2003). We suggested previously that p25 could compensate for learning and memory deficits when expressed at low level and – at the opposite – induce neurodegeneration when expressed at high level (Angelo *et al*., 2003). In this context it is interesting to note that**,** even if there is no evidence that p25 accumulates more in female brains than in male brains, the predisposition for Alzheimer's disease is higher for females than males (Rocca *et al*., 1991; Jorm & Jolley, 1998).

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Abbreviations

ACSF, artificial cerebro-spinal fluid; Cdk5, cyclin-dependent kinase 5; fEPSP, field excitatory postsynaptic potential; LTP, long-term potentiation; L-LTP, late-long-term potentiation; MAPK, mitogen-activated protein kinase; PPF, paired-pulse facilitation; WT, wild-type.

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