Chapter 5

Spatial, contextual and working memory are not affected by the absence of mossy fiber long-term potentiation and depression

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Abstract

The mossy fibers of the hippocampus display NMDA-receptor independent long-term plasticity. A number of studies addressed the role of mossy fiber long-term plasticity in memory, but have provided contrasting results. Here, we have exploited a genetic model, the rab3A null-mutant, which is characterized by the absence of both mossy fiber long-term potentiation and long-term depression. This mutant was backcrossed to 129S3/SvImJ and C57Bl/6 to obtain standardized genetic backgrounds. Spatial working memory, assessed in the eight-arm radial maze, was unchanged in rab3A null-mutants. Moreover, one-trial cued and contextual fear conditioning was normal. Long-term spatial memory was tested in the Morris water maze. Two different versions of this task were used, an "easy" version and a "difficult" one. On both versions, no differences in search time and quadrant preferences were observed. Thus, despite the elimination of mossy fiber long-term plasticity, these tests revealed no impairments in mnemonic capabilities. We conclude that spatial, contextual and working memory do not depend on mossy fiber plasticity.

Introduction

Granule cells of the dentate gyrus are a principal relay station of the hippocampus, receiving excitatory input from the enthorhinal cortex and projecting, by means of the mossy fibers, to area CA3. A number of findings have implicated granule cells in memory. For instance, granule cell lesions impair spatial memory (Walsh et al., 1986;Czeh et al., 1998), and the acquisition of spatial information is blocked by a reversible inactivation of mossy fiber neurotransmission (Lassalle et al., 2000). In addition, the size of the mossy fiber projection is positively correlated with performance in the eight-arm radial maze (Crusio et al., 1987). Finally, new granule cells are continuously generated in adults through neurogenesis, and not only are these new neurons important in trace-conditioning (Shors et al., 2001), but neurogenesis itself is enhanced by training in this hippocampus dependent learning task (Gould et al., 1999).

Much research has focused on the involvement of long-term potentiation (LTP) and long-term depression (LTD) in learning and memory. LTP and LTD are rapid and persistent changes in synaptic strength. NMDA-receptor dependent LTP and LTD are particularly well studied. A blockade of NMDA-receptor dependent plasticity by pharmacological (Morris et al., 1986) or genetic (Tsien et al., 1996) means impairs learning in the Morris water-maze and suggest an essential role for this type of synaptic plasticity in memory. Mossy fibers also display LTP (MF-LTP) and LTD (MF-LTD) but they are rather unusual. MF-LTP and MF-LTD are independent of the NMDA-receptor and expressed by changes in neurotransmitter release (Yamamoto et al., 1980;Zalutsky and Nicoll, 1990;Harris and Cotman, 1986;Staubli et al., 1990;Weisskopf and Nicoll, 1995;Kobayashi et al., 1996;Derrick and Martinez, Jr., 1996).

If MF-LTP and MF-LTD have a role in memory then changes in synaptic strength should occur during learning. Such measurements were done in rats. It was shown that CA3 field potentials, evoked by mossy fiber stimulation, increased in amplitude over the course of a few days of radial maze learning (Mitsuno et al., 1994;Ishihara et al., 1997). A number of genetic studies addressed the role of mossy fiber long-term plasticity in memory, but the results were not conclusive. Mice lacking type 1 metabotropic glutamate receptors (mGluR1) or type 1 adenylyl cyclase (AC1) have deficits in MF-LTP and are impaired in the Morris water maze (Conquet et al., 1994;Wu et al., 1995;Villacres et al., 1998). Other genetic models showed different results. Mice that lack the catalytic subunit Cβ1 or the regulatory subunit R1β of PKA also have no MF-LTP, but show normal Morris maze performance and normal contextual fear-conditioning (Huang et al., 1995). Furthermore, the mGluR2 null-mutant has normal MF-LTP but impaired MF-LTD and shows no deficits in the Morris maze (Yokoi et al., 1996).

Resolving this apparent contradiction is problematic for a number of reasons. Firstly, the genetic background of the mice in the studies was poorly defined. This will result in different genetic backgrounds of mutant and wildtype mice (Gerlai, 1996), and may have contributed to the apparently contrasting results. Secondly, the presence of MF-LTD was not tested in the mGluR1, AC1, Cβ1 and R1β mutants. It has been suggested that mossy fiber plasticity may be involved in reducing signal-to-noise ratios in granule cell signaling (Treves and Rolls, 1994). This might be achieved, at least to a certain degree, by using only MF-LTD and may be adequate to allow relatively normal hippocampal

functioning and normal cognitive performance. Thirdly, the mGluR1 mutant was also ataxic (Conquet et al., 1994), and the AC1 mutant also had impairments in NMDA-LTP in area CA1 (Wu et al., 1995). These deficiencies may have influenced performance in the learning tasks. Thus, the role of mossy fiber plasticity in learning and memory is unclear and new experiments are needed for a better understanding of this relationship.

In the present study, we analyzed a mutant that lacks both MF-LTP and MF-LTD, the rab3A null-mutant (Geppert et al., 1994). Rab3A is a neuron-specific protein, enriched in nerve terminals and implicated in the regulation of vesicular secretion (Sudhof, 1997). Rab3A deletion in mice does not affect basal transmission or short-term plasticity in the mossy fiber projection but eliminates both MF-LTP and MF-LTD (Castillo et al., 1997;Tzounopoulos et al., 1998). As far as we know, this is the only model with a simultaneous blockade of MF-LTP and MF-LTD. Mutants were tested in a variety of hippocampus-dependent learning tasks in order to measure different aspects of memory. Spatial working memory was tested in the eight-arm radial maze. Long-term spatial memory and spatial reversal learning were tested in the Morris water-maze. Contextual memory was tested in the fear-conditioning task. We report that rab3A null-mutants, although lacking mossy fiber long-term plasticity, show normal performance in all these tasks.

Materials and methods

Mice

Mutants were created by deleting the promoter and first two exons of the rab3A gene by homologous recombination (Geppert et al., 1994). Subsequently, mutants were repeatedly backcrossed to 129S3 (also known as 129/SvImJ; Jax code: JR2448) for at least 4 generations, resulting in a line with a standardized genetic background. A second line was obtained by backcrossing to C57Bl/6 for 4 generations. Mice were bred in our laboratory under standard conditions. At 25 days, they were weaned and housed with 2-4 mice of the same sex. Mice were kept at a 12h-light/dark cycle with lights on at 7:00 PM. Food (Hope Farm) and water were freely available. Null mutants were obtained by crossing heterozygotes. Wildtype littermates served as controls. The experimenter was always blind with respect to genotype until the end of the experiments. Mice were genotyped by PCR, which was occasionally confirmed by western blot. In all experiments, except for the Morris water maze, the backcross to 129S3 was used. The 129S3 strain is unsuitable for the Morris water maze because of its propensity to float (Wolfer et al., 1997). As recommended (Banbury Conference Report, 1997) we used the F1 between the 129S3 backcross and C57Bl/6 backcross. All experiments were approved by the Ethical Committee of the Utrecht Medical Center.

Electrophysiology

Transversal, 400μm-thick slices were prepared and kept in oxygenated (95% O_2, 5% CO_2) medium at room temperature for at least 60min, before being used. Medium composition in distilled H_2O (mM): 124.0 NaCl, 3.3 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 10.0 glucose, 20.0 $NaHCO_3$ and 2.5 $CaCl_2$. Recordings were done at 30°C. Bipolar stainless-steel stimulation electrodes (tip electrode diameter of 50μm),

insulated except for the tip, were placed at the afferent mossy fibers. Field excitatory postsynaptic potentials (fEPSPs), determined from the linear part of the trace, were recorded in the stratum radiatum of area CA3 with glass microelectrodes (tip diameter approximately 2μm). fEPSPs were quantified by determining the slope of the trace. The stimulus intensity was adjusted to evoke fEPSPs of half-maximum amplitude (at least 0.125mV), and kept constant thereafter. Stimulation frequency was 0.05Hz. After 15min of baseline recording, MF-LTP was induced with high-frequency stimulation (200Hz) for 1s, after which the responses were recorded for 60min.

Open field

The open field was a circular, moderately illuminated (80lux) arena constructed of gray PVC (diameter 78cm, walls 30cm high). The open field was divided in two parts, a circular central area with a diameter of 55cm and an edge area. Mice were placed in the edge area and allowed to freely explore the open field for the duration of 30min. The location of the mouse was recorded every 0.5s using Ethovision (Noldus technology, The Netherlands).

Eight-arm radial maze

The radial maze was made of transparent Plexiglas. All arms (23cm long, 6cm wide, 5cm high) were baited with 20mg food rewards (Noyes precision food pellets, P.J. Noyes Company Inc., Lancaster, New Hampshire, United States), which were placed behind a small barrier that prevented visual detection. At the end of each arm, food pellets were deposited behind a perforated wall. These pellets were not retrievable by the mice but supplied the arm with food odors that prevented detection of the real food rewards by olfaction. We followed the protocol of others (Schwegler et al., 1990). Twenty-four hours before training the animals were food, but not water, deprived. During training, they were kept at 85–90% of their pre-test bodyweight. Mice received 1 trial per day. The first day, they received a 10min habituation-trial in which they were allowed to freely explore the maze and gather 16 scattered food rewards. During the following four trials, only the ends of the arms were baited. Between successive arm visits, mice were confined in the central area for 5s by lowering guillotine doors located at the entrance of each arm. This procedure is known to disrupt chaining responses and kinesthetic strategies (Schwegler et al., 1990). Trials ended when all baits had been collected or when 15min had past, whichever came first. During later trials, all mice routinely took all baits within 15min. An error was defined as an entry with all four paws in a previously visited arm.

Morris water maze

The Morris maze was a circular white pool (diameter 132cm), filled with water (25°C) that was made opaque by adding small quantities of the white pigment Acoat X (Akzo Nobel, Sassenheim, The Netherlands) at a dilution of approximately 1:10.000. The platform was circular (diameter 15cm or 8cm) and made of perforated Plexiglas that provided the mice with extra grip. The platform was placed 1cm below the surface of the water in the center of a quadrant. To avoid visual orientation prior to release, the mice were transported in a white bucket from which they glided in the water towards the wall of the pool. Starting positions changed every trial in a pseudo-random fashion. A trial ended when a mouse was 10s on the platform or when 120s had passed. Mice that failed to find the platform within 120 s were lured to the platform with a wire mesh shovel, so that they climbed the platform by themselves and were left there for 10s. The shovel was also used as a means to retrieve the mice

from the platform in a stress-free manner. Their location was sampled every 0.1s using Ethovision (Noldus technology, The Netherlands). The first experiment used the 15cm platform. In this version of the task, mice received six trials a day with a 45-60 min interval between trials. At the start of the 4th day, the platform was transferred to the opposite quadrant. During the first 30s of trial 1 at day 4 preference for the old quadrant was determined (transfer test); mice that found the relocated platform in less then 30s were excluded from the analysis. At the end of the 5th day of training, the platform was removed and a probe test of 30s was given (7th trial).

In the second version of the Morris water maze, an 8cm platform was used. Mice received four training trials per day with an inter-trial interval of 30min, except for days 6 and 8 were they received three training trials. Probe tests, in which the platform was removed, were conducted 30min prior to training on day 1, 30min after training on day four, and 30min prior to training on days 6 and 8. Probe trials lasted 60s. The mice in the last study were used as a control group in a pharmacological experiment and received subcutaneous injections of saline (volume 10-15µl) 60min prior to training.

Contextual and cued fear conditioning

A transparent Plexiglas shock chamber (24*24 cm) with a stainless-steel rod floor (1 rod per 9mm, diameter rod 4mm) was used for training. The training protocol was as described by others (Bourtchuladze et al., 1994). Mice were placed in the chamber and allowed to habituate for 2 min before onset of the conditioned stimulus (CS: 30s, 85dB tone). During the last two seconds of the CS, the unconditioned stimulus (US: 0.75mA scrambled foot shock) was applied. After another 30s, the mice were returned to their home cage. The next day, mice were tested for contextual and cued fear conditioning. Mice were placed in the shock chamber (without CS) for a period of 5min in order to determine contextual fear conditioning. After a 2h interval, cued fear conditioning was assessed. Mice were placed in a novel cage for a period of 6min; during the last 3min, the tone CS was continuously applied. The strength of conditioning was determined by measuring the time spent freezing, which was defined as immobility with only an occasional head movement.

Statistics

The data were analyzed by t-tests for independent samples, and univariate and repeated measures of ANOVA. When the sphericity assumption was not met, Huynh-Feldt correction was applied.

Results

Rab3A mutant pups develop sensory motor skills normally, but have a lower bodyweight

All genotypes were represented according to a Mendelian distribution (wt:+/-: ko = 51:106:50). Null-mutants had a slightly lower bodyweight at weaning (at 21 days (mean±sem): mutants 9.1±0.3gr (n=11), wildtypes 10.1±0.4gr (n=12), $t(df=21)=1.90$, $p<0.01$). Mutants and wildtypes acquired sensomotoric skills at the same age (fore- and hind limb placing response, fore- and hind limb grasping response, stopping of pivoting, cliff drop aversion, straight-line walking, righting reflex, opening of the eyelids (data not shown)). This indicated that sensomotoric development was similar and that the reduced bodyweight did not represent a slower development of the rab3A mutants. In adult mice, bodyweight was not different (data not shown).

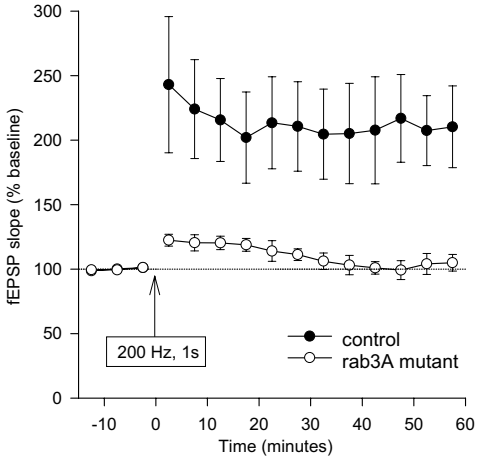

Figure 1. Changes in field EPSPs after tetanization (200Hz, 1s) of the mossy fiber projection. Mutant slices (n = 4) did not show potentiation, whereas control slices (n = 4) showed long-lasting increases in field EPSPs (average responses 30-60 min after tetanization (mean±sem): controls (heterozygotes) 209%±36, mutants 103%±5, t(df=6)=3.02, p<0.02).

Mossy fiber LTP is absent

The rab3A mutant was backcrossed to 129S3, resulting in a different genetic background than used in earlier studies. Therefore, MF-LTP was tested in these genetically standardized mice (Fig.1). Wildtype mice showed robust MF-LTP while Rab3A mutants failed (p<0.02). Thus, we confirmed earlier findings that showed the absence of MF-LTP in the rab3A mutant (Castillo et al., 1997).

Mutants lacking mossy fiber plasticity spend less time in the central area of the open field The open field test was used to study the mutant's response to a novel environment. The total test period was divided in three intervals. Mutants and wildtypes did not differ in traveled distances in the open field, and both groups showed a decline in traveled distance (Fig.2A). The time that mice spent in the

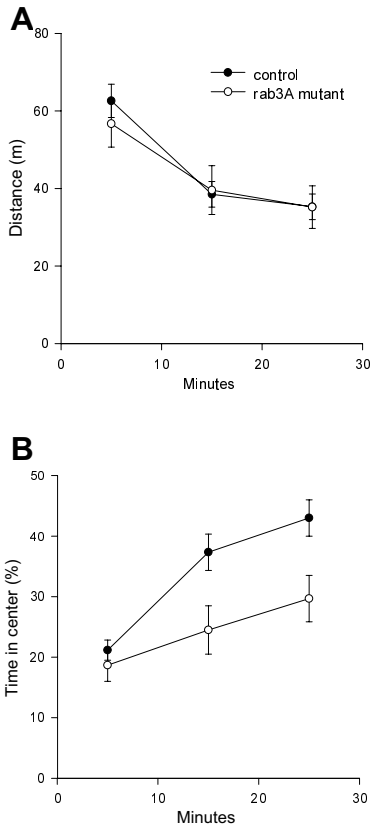

Figure 2. Open field behavior of wildtype (10 males) and mutant (10 males) mice. **A.** Traveled distance in 10min bins. Mutants and wildtypes did not differ (time*genotype: $F_{1.5,\ 27.1}$=1.63, n.s. (Huynh-Feldt corrected ε=0.75)), but both groups showed a decline in traveled distance over time (time: $F_{1.5,27.1}$=79.4, p<0.001, (Huynh-Feldt corrected ε=0.75)). **B.** Time in center in 10min bins. Wildtypes showed a stronger preference for the center at later intervals (genotype*time: $F_{2,36}$=5.41, p<0.01).

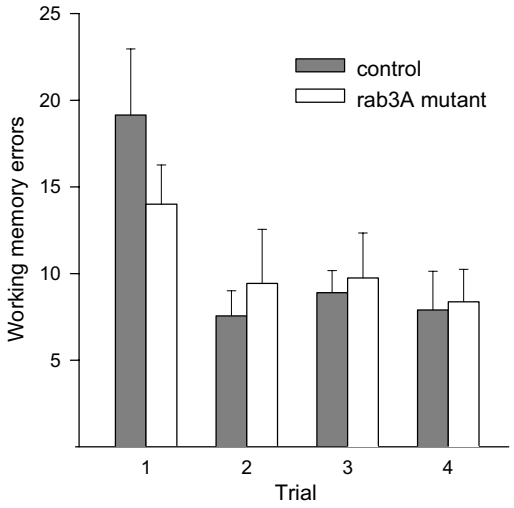

Figure 3. Radial maze performance of wildtype (10 males) and mutant (8 males) mice. The last four trials are shown. Mutants and wildtypes did not differ. Working memory errors in trial one: wildtypes 19.1±3.8, mutants 14.0±2.3, t(df=12)=1.16, n.s. Working memory errors at the last trial: wildtypes 7.9±2.2, mutants 8.4±1.9, t(df=16)=0.16, n.s.

central area increased at later intervals, but this was less in rab3A mutants ($p<0.01$) (Fig.2B). Thus, mutants showed a different response during habituation to a novel environment.

Working memory in the radial maze was not changed by the absence of mossy fiber plasticity

As a first aspect of memory formation we tested spatial working memory in the eight-arm radial maze. In this maze the ability to remember previously visited arms within the same trial is tested. The two groups showed a similar decline in working memory errors on consecutive trials (Fig.3). At the first day of training, wildtypes tended to make more working memory errors but this difference was not significant. Performance reached a plateau at the second trial in both groups and no further changes were detected on the third and fourth trial. Thus, mutants do not show impairments in this task indicating that MF-LTP and MF-LTD are not involved in working memory.

Spatial memory in the Morris water maze performance was not changed by the absence of mossy fiber plasticity

As a second aspect of memory formation we tested long-term memory for a specific spatial location using the Morris water maze. Two experiments were performed. In the first experiment a platform with a diameter of 15cm was used, and mice received 6 trials per day. During the first three days of training, the two groups showed a similar, rapid decline in search time (Fig.4A). Quadrant preference was determined during the first trial of day 4 (transfer test: Fig.4B). Controls and rab3A mutants showed a similar preference for the quadrant where the hidden platform used to be. Transfer of the platform resulted in increased search time on day 4, which was similar for both groups. At day 5, search time declined again. At the end of the fifth day, the platform was removed and quadrant preference was tested (probe test: Fig.4B). No differences were detected between controls and rab3A mutants. Furthermore, swimming speeds were not different between groups [during probe test at day 5: wildtypes 19±1 cm/s, mutants 21±1 cm/s, t(df=14)=1.6, n.s.]. In a second version of the Morris maze, task difficulty was increased by decreasing the platform size to 8cm and reducing the number of training trials to 4 per day. This version proved to be more difficult as evidenced by the longer search times (Fig.5A). Also in this difficult version of the Morris water maze, mutants performed comparably to

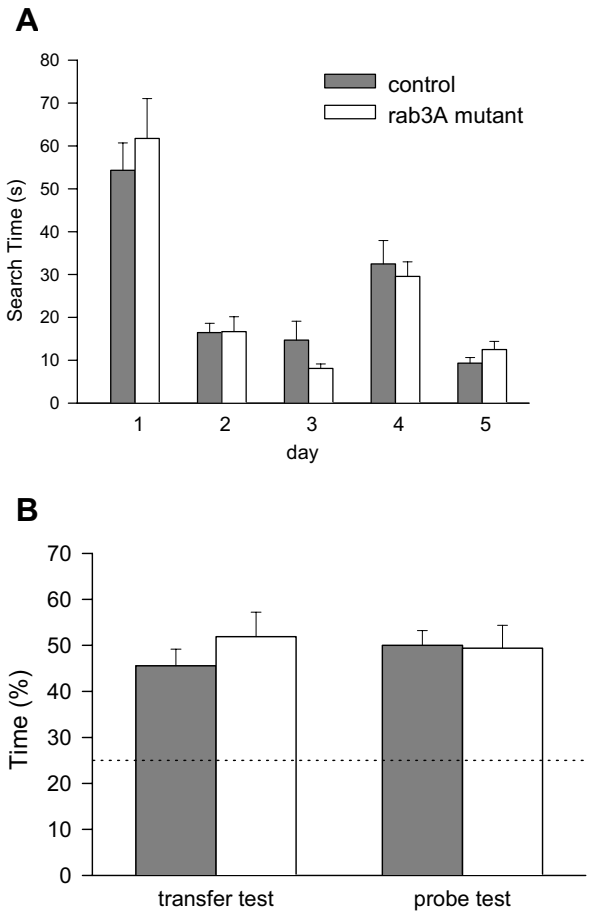

Figure 4. Morris maze "easy" version. Performance of control (wildtypes (4 males, 5 females) heterozygotes(3 males, 3 females) and null-mutant (3 males, 4 females) mice. **A.** Search time to find the hidden platform. Shown is the averaged search time of six daily trials. No main effects of sex or genotype nor interactions were observed (day*genotype: $F_{7.5, 59.6}$=0.67, n.s. (Huynh-Feldt corrected ε=0.93)). Heterozygotes and wildtypes were pooled, as were male and females. **B.** Search time (in percentage of total time) in the quadrant were the hidden platform used to be. No main effects or interactions were detected.

controls both in search times and quadrant preferences (Fig.5B). Thus, rab3A mutants performed normal in two versions of the Morris water maze, indicating that MF-LTP and MF-LTD are not involved in spatial learning and memory.

Contextual and cued fear conditioning was not changed by the absence of mossy fiber plasticity

As a third aspect of memory formation we tested contextual and cued fear conditioning using the association of a specific location (context) or a specific sound (cue) and an aversive foot-shock. It is believed that the strength of this association can be determined by measuring freezing behavior. The mice did not freeze during habituation in the first 2min or during the first presentation of tone CS (Fig.6A). This indicates that both the shock chamber and the tone CS had no *a priori* aversive properties. After the foot-shock was applied, both groups showed freezing responses. Contextual fear conditioning was assessed 24h later. During re-exposure to the conditioning chamber (Fig.6B) both groups showed similar amounts of freezing. Two hours later the mice were tested for cued fear conditioning (Fig.6C). When placed in the novel cage, the mice did not freeze, indicating that neither handling of the mice nor exposure to a novel cage elicited fear responses. During application of the tone CS, rab3A mutants and controls showed similar amounts of freezing. Again, rab3A mutants performed comparably to controls indicating that MF-LTP and MF-LTD are not involved in contextual and cued fear conditioning.

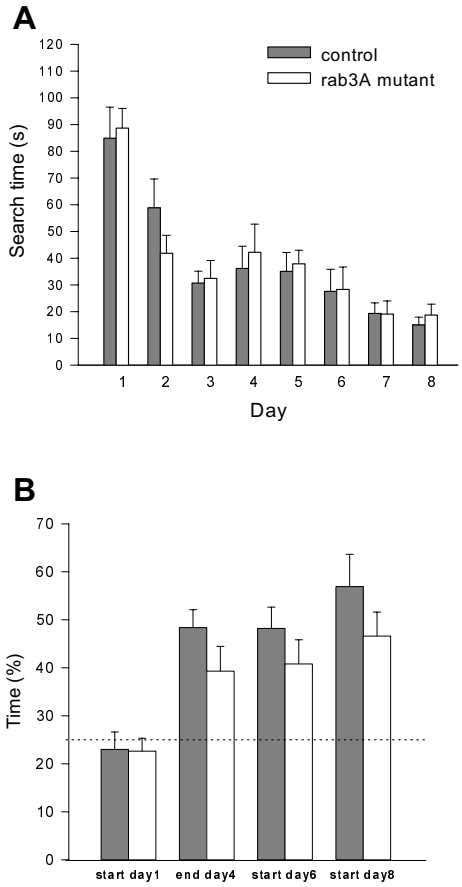

Figure 5. Morris maze "difficult" version. Performance of control (8 heterozygotes males) and null-mutant (10 males) mice. **A.** Search time to find the hidden platform. Shown is average of four daily trials, except for day 6 and 8, were only three training trials were given. Both groups showed a decline in search time (day: $F_{7,122}$ = 24.4, p<0.001), which did not differ between groups (day*genotype: $F_{7, 122}$ = 0.64, n.s). **B.** Search time (in percentage of total time) in the quadrant were the hidden platform used to be. The first probe test was before training, the second after training on day 4, the third and fourth were conducted before training on day 6 and 8. An increase in quadrant preference was observed for both groups (day: $F_{3,48}$=17.6, p<0.001), but no differences between mutants and controls were observed (day*genotype: $F_{3,48}$=0.56, n.s.).

Discussion

The aim of this study was to investigate the role of mossy fiber long-term plasticity in spatial and contextual memory. For this purpose we used rab3A null-mutant mice that have a deficit in both MF-LTP and MF-LTD. These mice were backcrossed in order to obtain a standardized genetic background and avoid the flanking region problem (Gerlai, 1996). The rab3A mutant performed normally in the radial maze, two versions of the Morris water maze and in the cued- and contextual fear-conditioning task. The only differences detected were a decrease in bodyweight in rab3A mutant pups and a minor change in open field behavior at later stages of that task.

This flanking region problem occurs when heterozygotes with a mixed genetic background (129/Sv and C57Bl/6) are used for breeding, which results in co-segregation of different regions flanking either the wildtype allele (derived from C57Bl/6) or the mutant allele (from 129/Sv). Mice that are hybrids of 129/Sv and C57Bl/6 outperform their parental strains in the Morris water maze (Owen et al., 1997), indicating that both inbred strains carry genetic polymorphisms that impair Morris water maze performance. Thus, differences in flanking regions may cause reduced or enhanced performance independent of the mutated gene. In the present study the backcross to 129S3 was used, which resulted in identical genetic backgrounds for mutant and control mice. The 129S3 inbred strain is

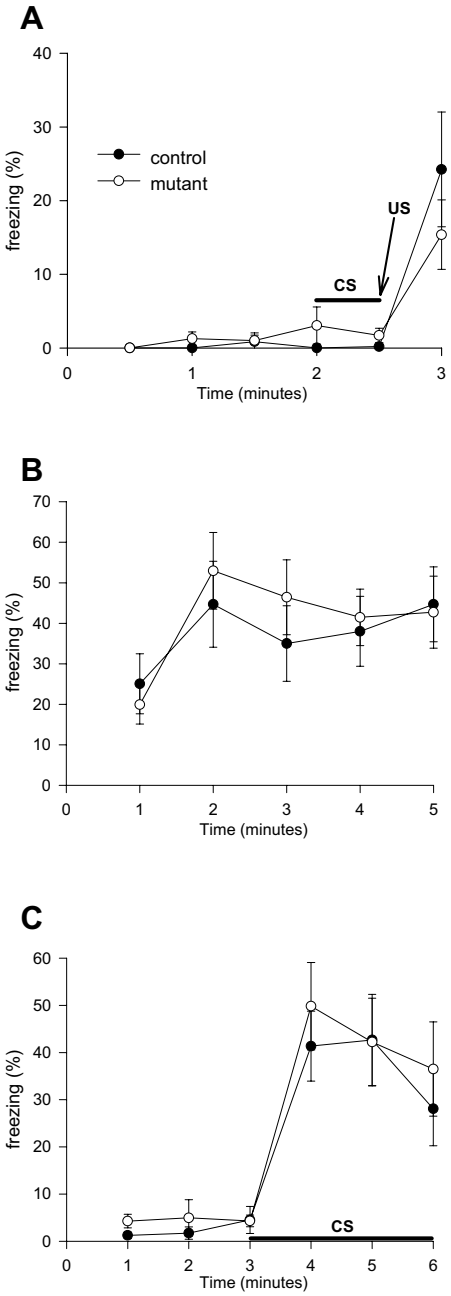

Figure 6. Cued and contextual fear-conditioning in wildtype (11 females) and mutant (12 females) mice. **A.** Freezing time in 30s bins during the training trial. Mutants and wildtypes did not show freezing during their first exposure to the shock chamber and the tone CS. After application of the foot shocks, both groups showed freezing responses that were similar (time: $F_{1.3,27.3}$=16.5, p<0.001 (Huynh-Feldt corrected ε=0.26)), (time*genotype: $F_{1.3,27.3}$=1.23, n.s. (Huynh-Feldt corrected ε=0.26)). **B.** Contextual fear-conditioning. Shown is freezing in 1min bins. Mutants and wildtypes freezed when placed in the shock chamber and showed similar levels of freezing (time: $F_{4,84}$=3.76, p<0.01), (time*genoype: $F_{4,84}$=0.45, n.s.), (genotype: $F_{1,21}$=0.16, n.s.). **C.** Cued fear-conditioning. Mice were placed in a novel environment were they were exposed to the tone CS after a 3min habituation period. During habituation, both groups showed virtually no freezing. After onset of the tone CS, both groups showed similar amounts of freezing responses (time: $F_{3.4,70.7}$=27.4, p<0.001 (Huynh-Feldt corrected ε=0.67)), responses (time*genotype: $F_{3.4,70.7}$=0.25, n.s. (Huynh-Feldt corrected ε=0.67)).

unsuitable for the Morris water maze because of its propensity to float (Wolfer et al., 1997), and the F1 between both backcrosses was used, as was recommended by others (Banbury Conference Report, 1997). In this way, the flanking region of only one chromosome differ and is also smaller in size, so that the genetic background problem is greatly reduced.

The results show that a complete elimination of long-term synaptic plasticity in the mossy fiber pathway does not impair learning and memory in a variety of tasks. This is surprising for a number of reasons. Firstly, the mossy fibers are an integral pathway in hippocampal information processing and granule cells share many firing characteristics with pyramidal cells of areas CA3 and CA1. Examples of such characteristics are spatially selective firing, theta precession, and reactivation of firing sequences during sleep (Jung and McNaughton, 1993;Skaggs et al., 1996;Shen et al., 1998).

Secondly, mossy fiber synapses are very large and believed to be powerful (Henze et al., 2000). Activity of only a few mossy fiber synapses could be sufficient to trigger a CA3 pyramidal cell (Henze et al., 2000). Thus, this pathway seems an ideal target to alter hippocampal information processing. Finally, mossy fibers show naturally occurring plasticity during radial maze learning, suggesting a role for mossy fiber plasticity in learning and memory (Mitsuno et al., 1994;Ishihara et al., 1997).

Our results do not exclude a subtle role for mossy fiber plasticity. The tasks that were used could all be learned within a limited number of trials and may not have been demanding enough to require mossy fiber plasticity. This issue was addressed using a difficult version of the Morris water maze. Even in this difficult version, mutants were not impaired. Apparently, other types of plasticity, such as those that are dependent on the NMDA-receptor are sufficient to ensure normal performance. Alternatively, synaptic plasticity in the mossy fiber pathway may not be important for the present task, but for future cognitive function. The dentate gyrus is one of the few regions in the brain that shows neurogenesis in adulthood. Interestingly, *in vivo* high-frequency mossy fiber stimulation, sufficient to induce MF-LTP, enhances neurogenesis (Derrick et al., 2000). Neurogenesis is also enhanced after a learning task (Gould et al., 1999) and in enriched environments (Kempermann et al., 1997) and these new neurons are necessary for trace conditioning (Shors et al., 2001). However, these new neurons need to be older than 6 days in order to be utilized in this learning task (Shors et al., 2001). Thus, mossy fiber plasticity may be involved in enhancement of neurogenesis, and consequently in learning and memory, but such effects will remain undetected in shorter learning tasks, such as those of the present study.

In summary, we tested the involvement of mossy fiber plasticity in learning and memory using a genetic model lacking both MF-LTP and MF-LTD. We circumvented the flanking allele problem by using appropriate breeding strategies and a selection of genetic backgrounds suitable for the different tasks. A small behavioral change was detected in the open field where mutants spent less time in the center. No differences were detected using the eight-arm radial maze, the Morris water maze and cued and contextual fear conditioning. Although we cannot exclude a subtle role for mossy fiber plasticity in memory, we can conclude that it is not essential for spatial, contextual and working memory.