Chapter 6

Complementary actions of two forms of long-term plasticity in spatial memory

Robert Hensbroek[a], Matthijs Verhage[a] and Berry Spruijt[a,b]

[a] *Rudolf Magnus Institute for Neurosciences*
Utrecht University Medical Center
Universiteitsweg 100
3584CG Utrecht
The Netherlands

[b] *Present address:*
Animal Welfare Center
Faculty of Veterinary Medicine
Utrecht University
Yalelaan 17
3584CL Utrecht
The Netherlands

Abstract

The hippocampus is essential for the acquisition of spatial memory and changes in synaptic strength are believed to be important for this process. The leading candidate mechanisms are NMDA-receptor dependent long-term potentiation and depression. NMDA-receptor dependent plasticity is displayed by most hippocampal pathways and its disruption by pharmacological or genetic means abolishes water-maze learning in naïve rodents. However, NMDA-receptor blockade fails to disrupt spatial memory in rats that have received drug-free pre-training in another room. Pre-training ensures familiarization with only the procedural requirements of the water maze. Thus, NMDA-receptor dependent plasticity does not appear essential for spatial learning. To resolve this issue, we blocked NMDA-receptor dependent LTP and LTD and hippocampal mossy fiber long-term plasticity. The latter types of plasticity are NMDA-receptor independent and absent in mice that lack rab3A. These mutants showed normal spatial memory during water-maze pre-training. Subsequently, they were trained in a novel room and simultaneously received the competitive NMDA antagonist (±)CPP at 10 mg/kg. Spatial memory was retained in wildtype mice, thus confirming earlier findings in rats. In contrast, mutants failed to show long-term spatial memory. These results suggest that NMDA-receptor dependent long-term plasticity and mossy fiber long-term plasticity can compensate for each other in a mutually exclusive fashion, and indicate that long-term synaptic plasticity in the hippocampus is both necessary and sufficient for long-term spatial memory.

Introduction

Spatial memory tasks such as the Morris water maze are widely used to assess mnemonic abilities in rodents. Spatial memory is believed to be an easily accessible analog for declarative memory in humans. Both declarative memory in humans and spatial memory in rodents are disrupted by lesions of the hippocampus (Milner et al., 1998;Morris et al., 1982;Bannerman et al., 1995). Much interest has focused on the physiological mechanisms involved. The foremost candidates are long-term potentiation (LTP) and long-term depression (LTD), rapid and persistent forms of synaptic plasticity. There are at least two kinds of long-term plasticity in the hippocampus, which can be distinguished on the necessity for NMDA-receptor activation. Many studies have emphasized the importance of NMDA-receptor dependent LTP and LTD (NMDA-LTP/LTD) in spatial memory. Disruption of NMDA-LTP/LTD by pharmacological (Morris et al., 1986) or genetic (Tsien et al., 1996) means abolishes water-maze learning in naïve rodents. However, in order to perform well in this task, animals must become familiar with the procedural requirements and suppress inappropriate behavior. Inadvertently, such aspects are included when studying naïve rats or mice. The problem is solved by water maze pre-training, which ensures the acquisition of the task requirements, and allows a refined assessment of spatial memory (Bannerman et al., 1995; Saucier and Cain, 1995). Using this procedure, it was shown that NMDA-receptor blockade fails to disrupt spatial memory in rats (Bannerman et al., 1995;Saucier and Cain, 1995). Thus, it seems that other mechanisms, independent of the NMDA-receptor, support spatial memory.

Most hippocampal pathways display NMDA-receptor dependent LTP and LTD, but there is an exception for the mossy fiber pathway from the dentate gyrus to area CA3. The mossy fibers display long-term plasticity (MF-LTP/LTD) that is not blocked by NMDA-receptor antagonists (Harris and Cotman, 1986;Kobayashi et al., 1996). The function of MF-LTP and LTD is unclear. They can be disrupted by gene manipulation, but that does not affect performance in a variety of learning tasks, including the water maze (chapter 5). Previously, we suggested that the loss of MF-LTP/LTD may be compensated by other types of plasticity (chapter 5).

Presently, this hypothesis was tested. To this end, we used rab3A null-mutant mice (Geppert et al., 1994). Rab3A is a presynaptic protein that is essential for MF-LTP and MF-LTD (Castillo et al., 1997;Tzounopoulos et al., 1998)(chapter 5). In combination with the NMDA-receptor antagonist (±)CPP, we were able to disrupt MF-LTP/LTD and NMDA-LTP/LTD and study the behavioral consequences. Our results show that spatial memory is preserved with a blockade of either NMDA-receptor dependent long-term plasticity or mossy fiber long-term plasticity. However, a simultaneous blockade of all these forms of long-term plasticity disrupts long-term spatial memory.

Material and Methods

Mice

Rab3A null-mutant mice were created by deleting the first two exons of the rab3A gene (Geppert et al., 1994). Mutants were repeatedly backcrossed to 129S3 (also known as 129/SvImJ; Jax code: JR2448) for at least 6 generations, resulting in a line with a standardized genetic background. A second line was obtained by backcrossing to C57Bl/6 for 6 generations. Heterozygotes for the rab3A gene from both lines were intercrossed resulting in hybrids which are known for their proficiency in the water maze (Wolfer et al., 1997;Banbury Conference Report, 1997). Wildtype and heterozygous littermates served as controls. 15 rab3A null mutant mice (5 males and 10 females) and 15 control mice (9 males and 6 females) were used for this experiment. All experiments were approved by the Ethical Committee of the Utrecht University Medical Faculty.

Morris water maze protocol

The Morris mazes were circular white pools (diameter 132 cm), 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 (15cm diameter), and made of perforated Plexiglas. The platform was placed 1 cm below the surface of the water in the center of a quadrant. To avoid visual orientation prior to release, 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. Their location was sampled every 0.1 s using Ethovision software (Noldus technology, The Netherlands). A trial ended when a mouse was 10 s on the platform or when 120 s 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 left there for 10 s. The shovel was also used as a means to retrieve the mice from the platform in a stress-free manner. Probe trials, in which the platform was removed, lasted 60 seconds. The training protocol is shown in table 1. The experimenter was always blind with respect to genotype until the end of the experiments. The two experimental rooms differed in size, orientation and dimensions. Animal cages were placed in different types of racks. The location of the door and the experimenter with computer setup was different. Finally, lightning of the room and spatial cues were different.

Table 1. Training protocol. Columns represent consecutive days, rows represent time after injection of (±)CPP (in minutes).

time \ day	1	2	3	4	5	6	7	8	9	10	11	12	13
0	-	-	-	-	-	-		c	c	c	c	c	-
60	p					p		p					p
90	t	t	t	t	t		room change	t	t	t	t	t	
120	t	t	t	t	t			t	t	t	t	t	
150	t	t	t	t	t			t	t	t	t	t	
180			p		p					p		p	

- no injection
c (±)CPP injection
t training trial
p probe trial

Treatment

In the second half of the experiment, mice received daily subcutaneous injections of the competitive NMDA-receptor antagonist (±)CPP [(±)–3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; Sigma RBI, Saint Louis, Missouri 63103, USA], at a dose of 10mg/kg. (±)CPP was dissolved in saline. The dose of 10 mg/kg disrupts *in vivo* dentate gyrus LTP in mice, until at least 150 minutes after injection (Davis et al., 1997), and primed-burst potentiation, an NMDA receptor dependent form of synaptic enhancement, in rats from 90-180 minutes after injection (Kentros et al., 1998). Mice received training trials from 90 to 150 minutes after (±)CPP injection. Thus, *in vivo* LTP is blocked by (±)CPP during the daily training sessions.

Statistics

Univariate ANOVA was used to analyze the data. In cases where it was inappropriate to use ANOVA, the Mann-Whitney U test was used. Spatial preference during probe tests was tested with one-sample T-tests against a test value of 25%.

Results

In the present study, experimental procedures were adapted from rats to mice. Rats are more adept in the Morris water maze so extra training is needed for mice. To assure thorough acquisition, mice received three training trails per day. Probe trials were used to measure spatial preference as these are considered the best measure for spatial memory (Lipp and Wolfer, 1998). The experimental protocol is shown in table 1. In short, mice received 5 days of drug-free pre-training. Subsequently, they were transferred to a new room and trained for another 5 days with NMDA-receptor blockade.

During drug-free pre-training, which was a standard Morris water maze procedure, all mice performed normal. Escape latencies declined during training (Fig.1A). Controls did not differ from rab3A mutants. The first probe test was performed at the start of the experiment. Mutants and controls did not show a spatial preference (Fig.1B), indicating that there was no bias towards the goal quadrant prior to training. The second and third probe tests were conducted 30 minutes after the last training trials on days 3 and 5. Little spatial preference was shown at day 3, but at day 5 both groups showed strong spatial preference (mutants $t(df=14)=4.8$, $p<0.001$; controls $t(df=14)=4.1$, $p=0.001$). The last probe test was performed one day after the last training in which both groups showed strong spatial preference (mutants $t(df=14)=4.0$, $p=0.001$; controls $t(df=14)=5.1$, $p<0.001$). Mutants never differed from controls during these probe tests. Thus, during pretraining, both groups showed short-term spatial memory as measured during the third probe trial and long-term spatial memory as measured in the fourth probe trial, and demonstrated their ability to solve the maze using spatial information.

After pretraining, mice were transferred to a new room, which had different dimensions and cues. Each day, prior to training, mice received an injection of the competitive NMDA antagonist (±)CPP (10 mg/kg). The protocol of the task did not differ from pre-training, hence mice were already familiar with the task requirements. This procedure should allow a specific assessment of spatial memory with as little interference of other aspects of Morris water maze learning as possible. Mice showed no signs of behavioral abnormalities under NMDA blockade, such as repeated deflection from the platform or

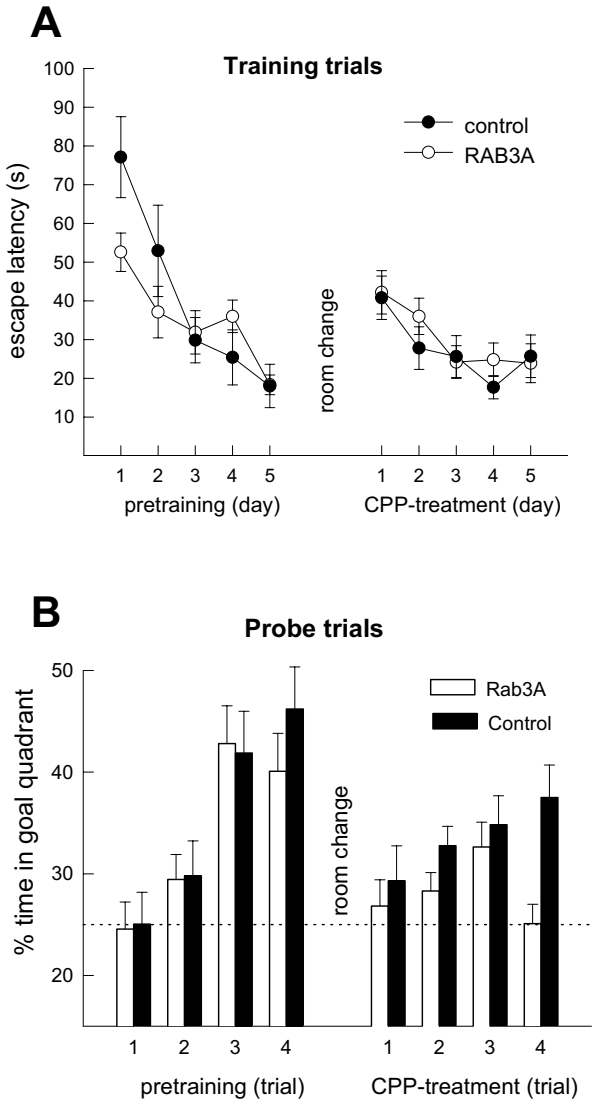

Figure 1. Morris maze performance of wildtype (n = 15) and mutant (n = 15) mice. **A.** Averaged escape latencies of 3 daily training trials expressed as mean±SEM. Shown at the left are the 5 pre-training days. At the right the 5 training days in the new room with simultaneous (±)CPP injections. No differences were detected **B**. Spatial memory was tested in probe tests during which the platform was removed. Preference for the goal quadrant was measured during 60 s free-swim trials. Preference was expressed as percentage of time in goal quadrant. Expressed as mean±SEM. Shown at the left are the 4 probe trials during pre-training. At the right the 4 probe trials in the new room with simultaneous (±)CPP injections. During both parts of the experiment, the patterns of probe trials were similar. Probe trial 1 was before training at day 1, probe trial 2 was 30 minutes after the last training trial of day 3, probe trial 3 was 30 minutes after the last training trial of day 5 and probe trial 4 was 1 day after the last training trial.

failures to climb the platform, a phenomenon that has been reported to occur in naïve rats, but not in pretrained rats (Li et al., 1997). At the first day of training, latencies were already short as compared to the first day of pre-training (Fig.1A). This shows that pre-training was effective in familiarizing the mice with the task. At later training days, latencies declined further, but controls were not different from mutants. The first probe trial (Fig.1B), given at the start of training in the new room, showed that there was no *a priori* preference for the goal quadrant. The second and third probe trial, taken 30 minutes after training on days 3 and 5 respectively, showed an increasing preference of control mice for the goal quadrant (2nd probe trial($df=14$)=4.1, $p=0.001$; 3th probe trial ($df=14$)=3.5, $p=0.004$). Mutants show only little spatial preference in the second probe trial, but demonstrated significant spatial preference in the third probe trial (2nd probe trial $t(df=14)=1.8$, $p=0.086$; 3rd probe trial $t(df=14)=3.1$, $p=0.007$). Mutants differed from controls at the 2nd probe trial ($F_{1,26}=5.9$, $p=0.023$), but not at the 3rd probe trial. Thus, both controls and mutants showed a preference for the goal quadrant immediately after training, thereby demonstrating short-term spatial memory, but mutants perform slightly worse

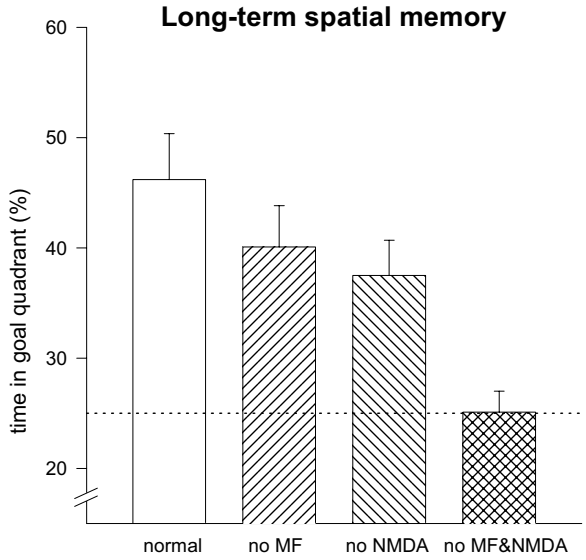

Figure 2. Summary of probe tests. Shown are results for long-term spatial memory during the last probe tests of each training phase.

then controls. The fourth probe trial, was taken 1 day after the last training trial. This probe trial measures long-term spatial memory. Control mice showed a strong preference for the goal quadrant ($t(df=14)=3.9$, $p=0.002$). Thus, control mice showed long-term spatial memory despite a blockade of NMDA receptors. Mutants, however, failed to show any spatial preference and differed from controls ($F_{1,26}=10.2$, $p=0.004$). Thus, when either mossy fiber long-term plasticity or NMDA-receptor dependent long-term plasticity is blocked, mice still display spatial memory, but under conditions where both forms of long-term plasticity are disrupted, mice fail to show long-term spatial memory in the Morris water maze (Fig.2).

Discussion

In the present study, we tested whether mossy fiber long-term plasticity and NMDA-dependent long-term plasticity can compensate for each other in spatial memory. To this end, we used rab3A null-mutant mice which lack both MF-LTP and MF-LTD. Mutants and controls were pre-trained under drug-free conditions and showed normal spatial memory. In the second phase of the experiment, mice were trained in a new room and were given the competitive NMDA antagonist (±)CPP at a dose that blocks *in vivo* NMDA-LTP. Controls and MF-LTP/LTD deficient mice did not differ in escape latencies nor did they differ on the first three probe trials. Both groups showed evidence of short-term spatial memory. The final probe trial, showed a strong spatial preference in controls, but mutants failed. Thus, mice, like rats (Bannerman et al., 1995;Saucier and Cain, 1995), are able to acquire and use spatial information with NMDA-receptor blockade. Our results suggest that in the absence of NMDA-receptor depedent plasticity, mossy fiber long-term plasticity is able to compensate.

In the present study, we used a platform of 15 cm in diameter and a pool size of 135 cm in diameter. Thus, compared to pool size, the platform size was relatively large and enabled mice to find the platform by random swim patterns. Indeed, only 3 out of 30 mice failed to find the platform during the

first training trial of the second phase. An advantage of such a setup is that potential problems such hypothermia and learned helplessness are kept minimal, and that mice are able to locate and climb the platform even when they are not aware of its spatial location. A disadvantage is that it may be hard to detect differences in escape latencies, such as in the present study. However, the only reliable way to measure spatial memory is with probe tests, because a probe test can distinguish between a spatial search strategy and a non-spatial search strategy (Lipp and Wolfer, 1998). These probe tests showed that mutants treated with (±)CPP do show a spatial preference 30 minutes after training. Hence, under conditions where both NMDA-LTP/LTD and MF-LTP/LTD are blocked, spatial memory is still supported, but this is only transient, and lost within one day.

The hippocampus has been specifically implicated in spatial learning because neurons of the hippocampus show spatially selective firing. This finding has led to the proposal that the hippocampus can support a spatial map of the environment that animals can use to navigate (O'Keefe and Dostrovsky, 1971). Spatially selective firing develops rapidly in a new environment and remains stable for many days (O'Keefe and Dostrovsky, 1971;Muller et al., 1987). Spatially selective firing is also observed in the water maze (Hollup et al., 2001). The long-term stability of these “place cells” can be affected by NMDA-receptor blockade (Kentros et al., 1998). However, spatial navigation is not blocked by NMDA-receptor blockade after pre-training (Bannerman et al., 1995;Saucier and Cain, 1995), which suggests a dissociation between the long-term stability of place cells and long-term spatial memory. However, place cells are usually measured in a pellet chasing tasks where spatial cues have little behavioral significance. Even under these circumstances, place cells tend to cluster around objects and along walls (Eichenbaum et al., 1999), which indicates that the “spatial map” concentrates around cues that may have some significant behavioral value. Thus, brain systems that are implicated in emotion and arousal may be involved in creating the spatial map. A target for such modulation of the spatial map could be provided by the mossy fibers. The terminal region of the mossy fibers also receives an extensive noradrenergic projection from the locus coeruleus (Loy et al., 1980). The induction of MF-LTP requires activation of β-adrenergic receptors (Huang and Kandel, 1996). Thus, simultaneous activity of hippocampal granule cells and activity in noradrenergic fibers appear necessary to induce long-term plasticity at the mossy fiber terminals (Bailey et al., 2000). As the locus coeruleus becomes active in situations of behavioral arousal (Huang and Kandel, 1996), it appears likely that noradrenergic activity is heightened in the life-threatening water maze. This way, mossy fibers may be able to support a spatial map in behaviorally challenging situations such as the water maze, but not in spatially undemanding situations such as a pellet chasing task.

The mossy fibers are suitable for such a role in spatial memory because mossy fiber synapses are large, have multiple active zones and believed to be very powerful. Activity in only a few mossy fiber synapses may be sufficient to depolarize a CA3 pyramidal cell above threshold (Henze et al., 2000). Also, granule cells show spatially selective firing (Jung and McNaughton, 1993). Thus, expression of MF-LTP and MF-LTD could strongly influence the spatial firing characteristics of CA3 pyramidal cells and consequently influence hippocampal function in spatial navigation.

In summary, our results suggest that short-term spatial memory is supported by transient mechanisms independent of mossy fiber long-term plasticity and NMDA-dependent long-term plasticity. The

absence of either NMDA-plasticity or mossy fiber long-term plasticity does not prevent long-term spatial memory. However, a blockade of both forms of long-term plasticity disrupts long-term spatial memory, which indicates that NMDA-plasticity and mossy fiber long-term plasticity are able to compensate for each other.