Chapter 3

Disruptions of presynaptic plasticity do not affect spatial memory but cause hyperactivity and impair working memory performance

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Abstract

Learning and memory are believed to be mediated by changes in synaptic strength. Synaptic strength can be modulated by changes in neurotransmitter release, but the importance of such changes for learning and memory is not clear. Here, this relationship was tested using munc18-1 gene-dose (or heterozygous) mutant mice that have impairments in presynaptic plasticity. Previously, it was shown that munc18-1 gene-dose mutants show a reduction of munc18-1 protein levels throughout the brain. Such a decrease of munc18-1 does not affect basal transmission and paired-pulse facilitation but causes an enhancement of tetanic depression and a disruption of post-tetanic potentiation and mossy fiber LTP. Despite these severe impairments in presynaptic plasticity, we observed no deficits in the Morris water maze. The eight-arm radial maze was used to assess spatial working memory. Mutants showed no improvements in this task and remained at pre-training levels. Thus, these forms of presynaptic plasticity may be involved in working memory, but are not important for spatial memory. Munc18-1 gene-dose mutants showed a higher locomotor activity in the radial maze. Such increases in locomotor activity were also observed in the open-field. The sensitivity to psychostimulants was studied with d-amphetamine, which strongly increased locomotor activity in mutants compared to wildtypes. We conclude that tetanic depression, post-tetanic potentiation and mossy fiber LTP are not important for spatial memory, but may be necessary for working memory. Disruption of presynaptic plasticity does not lead to selective effects on learning, but also to other deficits such as hyperactivity.

Introduction

Learning and memory are believed to be mediated by modifications in synaptic strength, but the exact mechanisms are not clear. One of the mechanisms involved may be a change in neurotransmitter release. Neurotransmitter release can be altered by short-term enhancements such as facilitation, augmentation and post-tetanic potentiation (PTP), but also by different types of short-term depression (Goda and Sudhof, 1997;Fisher et al., 1997;Zucker, 1999;Thomson, 2000b). Furthermore, neurotransmitter release can be altered for longer periods, such as occurring during long-term potentiation (LTP) and long-term depression (LTD at the mossy fiber projection of the hippocampus (Yamamoto et al., 1980;Harris and Cotman, 1986;Staubli et al., 1990;Weisskopf and Nicoll, 1995;Kobayashi et al., 1996). These changes in neurotransmitter release may be important in learning and memory, but this hypothesis has not been studied extensively due to the difficulty of manipulating neurotransmitter release in freely moving animals. Recently, a number of studies have appeared that used genetically manipulated mice to study the function of presynaptic plasticity in learning and memory, but the result were inconclusive. For instance, synapsinII null-mutants and synapsinI/II doubly mutants show lowered PTP and display mild deficits in contextual fear conditioning (Silva et al., 1996). The function of mossy fiber long-term potentiation (LTP) was tested in mGluR1 and type 1 adenylyl cyclase (AC1) null-mutants, which show impaired mossy fiber LTP and impaired performance in the water maze (Conquet et al., 1994;Wu et al., 1995;Villacres et al., 1998). However, other studies failed to relate deficits in presynaptic plasticity to memory impairments. For instance, rab3A null-mutants show enhanced paired-pulse facilitation, stronger tetanic depression and an abolishment of both mossy fiber LTP and LTD (Geppert et al., 1994;Geppert et al., 1997;Castillo et al., 1997;Tzounopoulos et al., 1998). Despite these impairments of presynaptic plasticity no deficits were observed in contextual fear conditioning, water maze learning and radial maze performance (chapter 5). Also, mice that lack a catalytic subunit (Cβ1) or a regulatory subunit (RIβ) of PKA have no MF-LTP but show normal Morris maze performance and normal contextual fear-conditioning (Huang et al., 1995). Thus, although a number of efforts have been made, the role of short and long-term presynaptic plasticity in learning and memory is unclear, and further studies are needed.

Presently, this relationship was tested using munc18-1 gene-dose mutant mice. Munc18-1 is expressed in all neurons (Garcia et al., 1994), and is essential for neurotransmitter release (Verhage et al., 2000). Gene-dose mutants (or heterozygotes) lack one copy of the gene for munc18-1 and are viable. Previously, it was shown that these mutants show a reduction in munc18-1 protein levels throughout the brain, which does not affect basal transmission and paired-pulse facilitation but causes an enhancement of tetanic depression, a disruption of post-tetanic potentiation and a severe impairment in mossy fiber LTP (chapter 2). The learning capabilities of these mutants were tested in the Morris water maze and the radial maze. Our results show that spatial learning was normal but that working memory performance was severely impaired. Mutants also showed a marked increase in locomotor activity.

Materials and methods

Mice

Mutants were created by replacing exon 2-5 of the Munc18-1 gene with a Neomycin resistant gene (Verhage et al., 2000). 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. Mice were bred in our laboratory under standard conditions. At three weeks, 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 was freely available.

Gene-dose mutant and wildtype experimental animals were obtained by crossing heterozygote males with 129S3 females. For the Morris water maze task, heterozygote males were crossed with C57Bl/6 female to obtain a C57Bl/6-129S3 genetic background. This breeding strategy was used because 129S3 mice have a propensity to show excessive floating in the Morris water maze, while C57Bl/6-129S3 hybrids perform well in this task (Wolfer et al., 1997;Banbury Conference Report, 1997). This breeding scheme was also used to study open-field behavior on different genetic backgrounds. The experimenter was always blind with respect to genotype until the end of the experiments. Mice were genotyped by PCR. All experiments were approved by the Ethical Committee of the Utrecht University Medical Faculty.

Developmental measures

Seven sensorimotor responses and bodyweight were recorded daily from day 3 to day 21. The age of appearance of the following responses were measured. Cliff drop aversion was shown when the pup turns around and crawls away when its head is placed over the edge of a tabletop. Hindlimb placing is displayed when the pup raises its limb and places it on top of a pencil when the front of hindlimb paw is touched with that pencil. Forelimb grasp is shown when a rod is grasped when the inside of the forelimb paw is touched with that rod. Stop pivoting is displayed when the pup does not pivot around anymore when placed on a tabletop. Rightning reflex is shown when the pup turns around within 5s after being placed on its back. Straight line walking is displayed when the pup walks away in a straight line when placed on a tabletop. Eye opening is shown when both eyes are fully opened.

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) 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. The training protocol was essentially the same as described by others (Wolfer et al., 1997). 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. Their location was sampled every 0.1s using Ethovision software (Noldus technology, The Netherlands). A trial ended when a mouse was 10s on the platform or when 120s had passed. The mice received six trials a day with a 45-60min 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.

Eight-arm Radial Maze

The radial maze was made of transparent Plexiglas. All arms (23cm long, 6cm wide, 5cm high) were baited with food rewards (20mg, Noyes precision food pellets, P.J. Noyes Company Inc.), which were placed behind a small barrier (1cm high, 1 cm long) 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. A standard protocol was used (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. On the following 8 days, between arm visits, mice were confined for 5 s in the central area by lowering guillotine doors located at the entrance of each arm. This procedure is known to disrupt chaining responses and kinesthetic strategies. Trials ended when all baits had been eaten 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.

Open Field

The open field task was used to study general measures of activity. 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 central area with a diameter of 55cm and an outer ring. Mice were placed in the outer ring and allowed to freely explore the open field for the duration of 15min. The location of the mouse was recorded every 0.5s using Ethovision (Noldus technology, The Netherlands).

Amphetamine

Mice received subcutaneous injections of d-Amphetamine (OGP, Utrecht, The Netherlands), at a dose of 0, 1 and 4 mg/kg bodyweight. Immediately after injection, they were placed in an observation cage (48cm long, 24cm wide, 24cm), that was moderately illuminated (80lux). Locomotor activity was monitored for 60min thereafter, by recording the location of the mouse every 0.5s using Ethovision (Noldus technology, The Netherlands).

Statistics

Non-parametric data was analyzed with the Chi-square test and Mann-Whitney-U test. Parametric data was 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

Munc18-1 gene-dose mutants have lower bodyweights but showed normal sensorimotor development

Mutants appeared normal upon visual inspection. At weaning, genotypes were represented according to a mendelian distribution (Table 1). However, munc18-1 gene-dose mutant pups had a lower

Table 1 Genotype frequency, bodyweight and sensorimotor development. Shown are means±sem.

	Wildtype	M18 +/-	Statistics
Genotype frequency	155	135	χ^2(df=1)=1.38, n.s.
Bodyweight			
Males at 21 days (g)	9.1±0.9 (n=4)	8.6±0.3 (n=6)	Sexe: $F_{1,20}$=0.78, n.s.
Females at 21 days (g)	10.2±0.3 (n=3)	7.8±0.2 (n=11)	Genotype: $F_{1,20}$=12.0, p<0.005
Males at 84 days (g)	28.7±2.1 (n=4)	26.2±0.5 (n=6)	Sexe: $F_{1,19}$=50.9, p<0.001
Females at 84 days (g)	23.2±0.6 (n=3)	19.4±0.3 (n=10)	Genotype: $F_{1,19}$=13.3, p<0.005
Sensorimotor development	n=7	n=17	
cliff drop aversion (day)	8.0±0.4	7.5±0.2	MWU=41.5, n.s.
hindlimb placing (day)	8.1±0.3	8.5±0.2	MWU=46.0, n.s.
forelimb grasp (day)	9.1±0.5	9.2±0.2	MWU=53.0, n.s.
stop pivoting (day)	10.7±0.8	11.7±0.3	MWU=42.0, n.s.
rightning reflex (day)	11.3±0.7	12.8±0.2	MWU=33.0, n.s.
straight line walking (day)	11.6±0.4	11.9±0.4	MWU=54.5, n.s.
eye opening (day)	13.6±0.4	13.4±0.2	MWU=50.5, n.s.

bodyweight at weaning (p<0.005). Such a decrease in bodyweight may result in retarded development, so next we determined the age of appearance of a variety of sensorimotor abilities. No differences were detected, indicating that the lowered bodyweight did not affect general development (Table 1). As adults, gene-dose mutants also had a lower bodyweight (p<0.005). General sensorimotor ability in adults was tested on a rotarod. No impairments in munc18-1 gene-dose mutants were detected (data not shown).

Spatial learning and memory in the Morris water maze are normal

The Morris water maze was used to assess long-term spatial reference memory. During the first three days of Morris maze training, both groups showed a rapid decline in search time (Fig.1A). At the first day, search time in wildtypes appeared higher compared to mutants, but this difference was not significant (t(df=22)=-1.74, p>0.1). Transfer of the platform to the opposite quadrant resulted in an increased search time on day 4, which returned to lower levels on day 5, indicating the ability of mutants and wildtypes to relearn a new platform location. Spatial memory was determined during the transfer test on day 4 and during the probe test on day 5. Wildtypes and munc18-1 gene-dose mutants showed a strong preference for the quadrant were the hidden platform used to be (Fig.1B), both during the transfer test at day 4, and during the probe test at day five. These results show that long-term

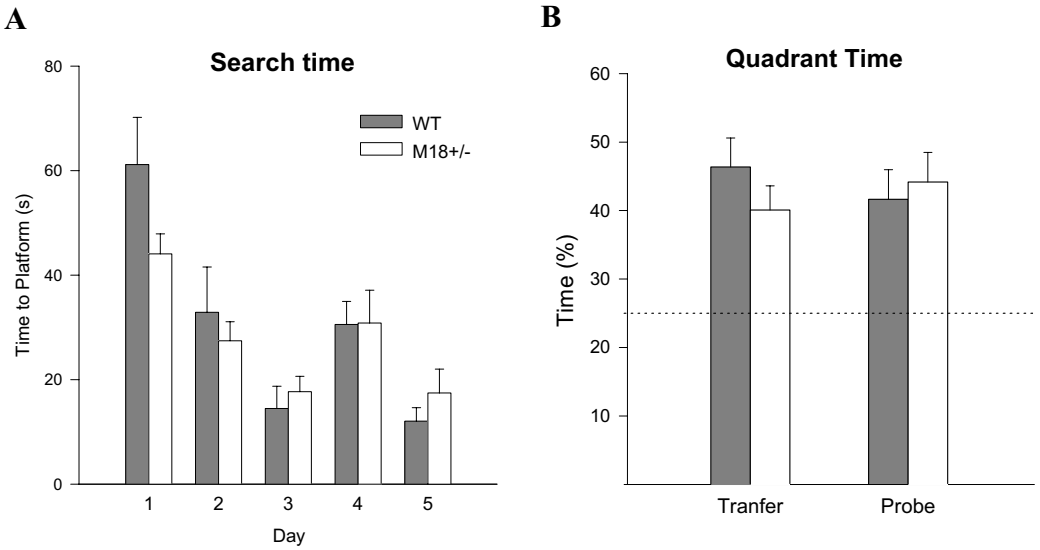

Figure 1. Morris maze performance of wildtypes (6 male and 6 female mice) and gene-dose mutants (5 male and 7 female mice). **A.** Search time to find the hidden platform. Shown is average of six daily trials. No significant main effects or interactions were detected: day*genotype*sex $F_{3.3,66.5}$=1.75, p=0.16 (Huyhn-Feldt corrected ε=0.83); day*sex $F_{3.3,66.5}$=1.71, p=0.17 (Huyhn-Feldt corrected ε=0.83); day*genotype $F_{3.3,66.5}$=2.43, p=0.067 (Huyhn-Feldt corrected ε=0.83); genotype $F_{1,20}$=0.03, n.s.; sex: $F_{1,20}$=0.77, n.s. The trend in the interaction day*genotype was attributable to search times on day 1 were wildtypes showed somewhat longer latencies than munc18-1 gene-dose mutants. **B.** Search time (in percentage of total time) in the quadrant were the hidden platform used to be. No differences were detected at day 4: wildtype n = 5 male and 3 female mice, gene-dose mutants n = 2 male and 4 female mice (genotype*sex: $F_{1,10}$=2.15, p=0.17; sex: $F_{1,10}$=0.38, n.s.; genotype: $F_{1,10}$=0.54, n.s.), and at day five: wildtype n = 6 male and 6 female, gene-dose mutants n = 5 male and 7 female. (genotype*sex: $F_{1,20}$=0.13, n.s.; sex: $F_{1,20}$=0.26, n.s.; genotype: $F_{1,20}$=0.14, n.s.).

spatial memory is not affected by lowered levels of munc18-1 and indicate that PTP and mossy fiber LTP are not essential for acquisition and retrieval of spatial information.

Performance in the radial maze is severely impaired

The eight-arm radial maze was used to assess spatial working memory i.e. the ability to remember previously visited arms within the same trial. Fig.2A shows the number of errors over the course of 8 days of training. Wildtypes showed a decline in the amount of working memory errors that reached a plateau after approximately 4 trials. In contrast, mutants failed to show improvements in their performance. A number of significant effects were detected, including a main effect for genotype ($p<0.01$) and a trial*genotype effect ($p<0.05$). Thus, munc18-1 gene-dose mutants performed worse than wildtypes. The accumulation of errors is shown in Fig.2B. Gene-dose mutants seemed to accumulate most errors during the collection of the last baits ($p<0.06$). At that point working memory load is highest, which indicates that the difference between mutants and wildtypes may have been the result of a decreased ability to maintain and use larger amounts of information in working memory.

Munc18-1 gene-dose mutants show increased activity

While conducting the radial maze task, it was noticed that mice that performed poorly seemed to have a higher locomotor activity. Post-hoc analysis revealed that these mice were mutants. Mutants spend less time in an arm that was previously visited ($p<0.01$), but were comparable to wildtypes when an arm was visited for the first time (Table 2). Swimming speeds in the Morris water maze were not changed (Table 2), and it is possible that increased activity may have selectively interfered with radial

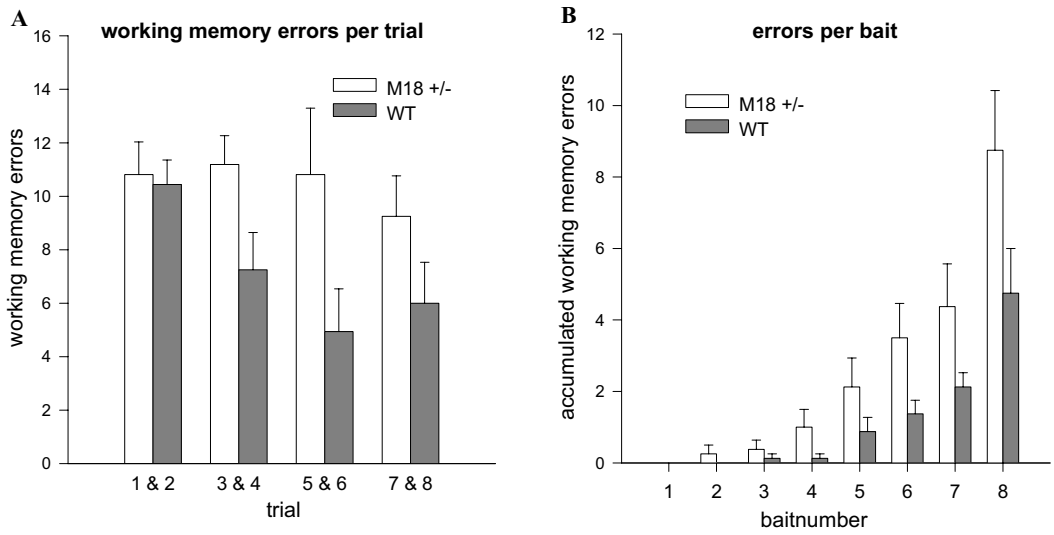

Figure 2. Radial maze performance of wildtype (5 male and 3 female mice) and mutant (3 male and 5 female mice) mice. **A.** Average errors per two trials. Statistical analysis showed significant interaction effects for: trial*genotype ($F_{3,36}$=3.88, p<0.05) and trial* sex ($F_{3,36}$=6.46, p<0.005). Also, a significant main effect was detected for genotype ($F_{1,12}$=9.56, p<0.01), whereas sex showed a trend ($F_{1,12}$=4.58, p=0.054). **B.** Accumulation of errors during the last trial. Shown are the number of accumulated errors before the next bait is found. The interaction baitnumber*genotype showed a trend ($F_{2.79,33.5}$=2.71, p=0.064 (Huynh-Feldt corrected ε=0.40)). There was a significant main effect for genotype ($F_{1,12}$=6.24, p<0.05). No interactions or main effect for sex were detected.

maze performance. In order to further analyze this increased activity, the open field task was used, which provides general measures of exploratory activity. Different genetic backgrounds were used in the radial maze (129S3) and the Morris water maze (C57Bl/6-129S3). Therefore, both genetic backgrounds were tested in the open field. In this task, gene-dose mutants walked much larger distances then wildtypes (p<0.001)(Fig.3A), which was mainly attributable to fact that mutants showed only little inactivity (p<0.001)(Fig.3B). Wildtypes 129S3 mice showed lower levels of activity than wildtype C57Bl/6-129S3 mice (wildtype only: strain effect $F_{1,24}$=27.6, p<0.001). The time in the central area was used as a measure of dispersion in the open field (Fig.3C). In the 129S3 genetic background, mutants and wildtypes differed (129S3 only: genotype*time effect: $F_{1.4,32.2}$=7.13, p<0.01 (Huyhn-Feldt corrected ε=0.70)). However, mutants and wildtypes did not differ when they had a

Table 2 Measures of activity in the radial maze and Morris water maze. Running speed in the radial maze was determined during the last trial, visits to empty arms were separated from visits to arms that still contained food rewards. Swimming speed in the Morris maze was determined during the last probe trial. Shown are means±sem.

	Wildtype	M18 +/-	Statistic
Radial maze	n=8	n=8	
Time in baited arm (s)	24.1±2.0	23.1±2.8	t(df=14)=0.47, n.s.
Time in empty arm (s)	25.3±3.7	11.7±1.2	t(df=14)=3.38, p<0.01
Morris maze	n=12	n=12	
Swim speed (cm/s)	18±1.0	17±1.0	t(df=22)=1.70, n.s.

C57Bl/6–129S3 background. Thus, munc18-1 gene-dose mutants showed enhanced activity in the

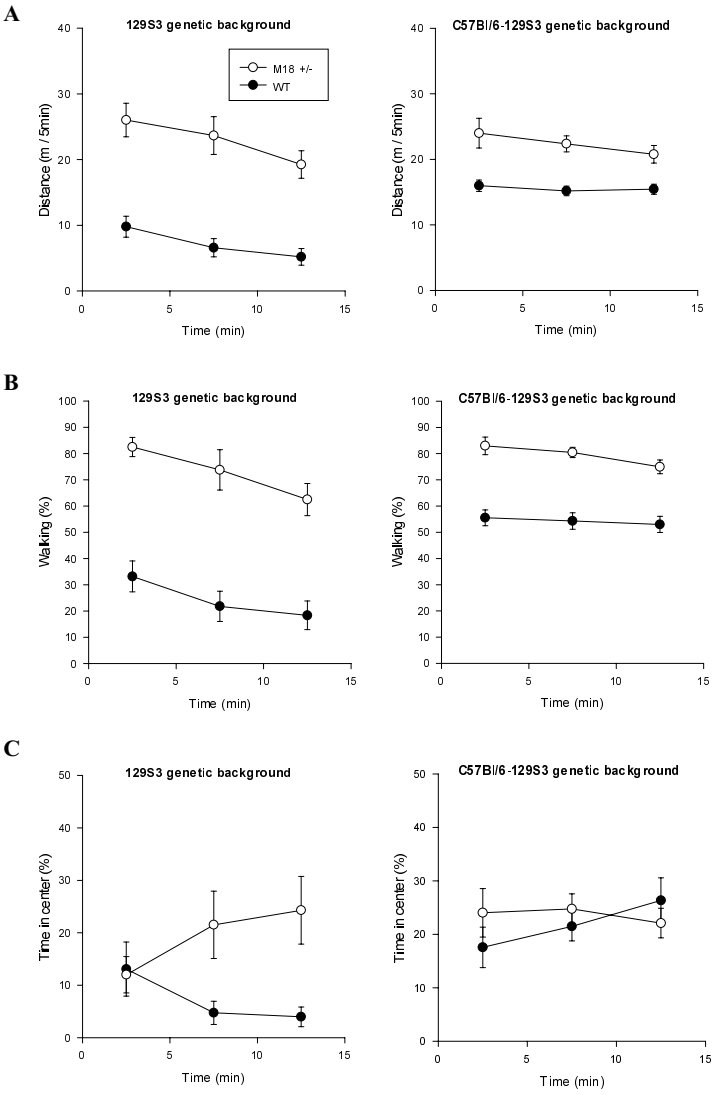

Figure 3. Open field behavior. 129S3 genetic background: gene-dose mutants (5 male and 5 female mice) and wildtypes (9 male and 8 female mice); C57Bl/6-129S3 genetic background: gene-dose mutants (5 male and 5 female mice) and wildtypes (6 male and 5 female mice). **A**. Traveled distance in 5min bins. Significant effects: time*strain ($F_{2,80}$=3.91, p<0.05), genotype*strain ($F_{1,40}$=9.54, p<0.005), strain ($F_{1,40}$=7.17, p<0.05) and genotype ($F_{1,40}$=60.9, p<0.001). **B**. Time spent moving in 5min bins. Significant effects: time*strain ($F_{2,80}$=3.79, p<0.05), genotype*strain ($F_{1,40}$=6.92, p<0.05), strain ($F_{1,40}$=16.7, p<0.001) and genotype ($F_{1,40}$=69.1, p<0.001). **C.** Time in center in 5min bins. Significant effects: time*sex*genotype*strain ($F_{1.94,77.6}$=4.58, p<0.05 (Huynh-Feldt corrected ε=0.97)), time*genotype*strain ($F_{1.94,77.6}$=8.62, p<0.001 (Huynh-Feldt corrected ε=0.97)), strain ($F_{1,40}$=8.21, p<0.01) and genotype ($F_{1,40}$=4.34, p<0.05).

open-field, which was mainly caused by incessant walking. Interestingly, the phenotype of mun c18-1 gene-dose mutants persisted in both genetic backgrounds, and genetic background had surprisingly little influence on measures of open-field behavior in mutants. In contrast, wildtype scores were much more dependent on genetic background, which shows the need for well-defined genetic backgrounds in tasks such as the open-field.

Munc18-1 gene-dose mutants are suspectible to d-amphetamine

To characterize the increased locomotor activity of munc18-1 gene-dose mutants, their response to d-amphetamine (AMPH) was studied. AMPH is a psychostimulant that is well known for its ability to increase random, non-directed locomotor activity. Paradoxically, in some animal models of hyperactivity and in patients with attention deficit hyperactivity disorder (ADHD), psychostimulants like AMPH are able to reduce symptoms of inattentiveness, hyperactivity and impulsivity (Solanto, 1998).

Mice were injected with saline, 1 and 4 mg AMPH per kg bodyweight. Injection of 4 mg/kg AMPH

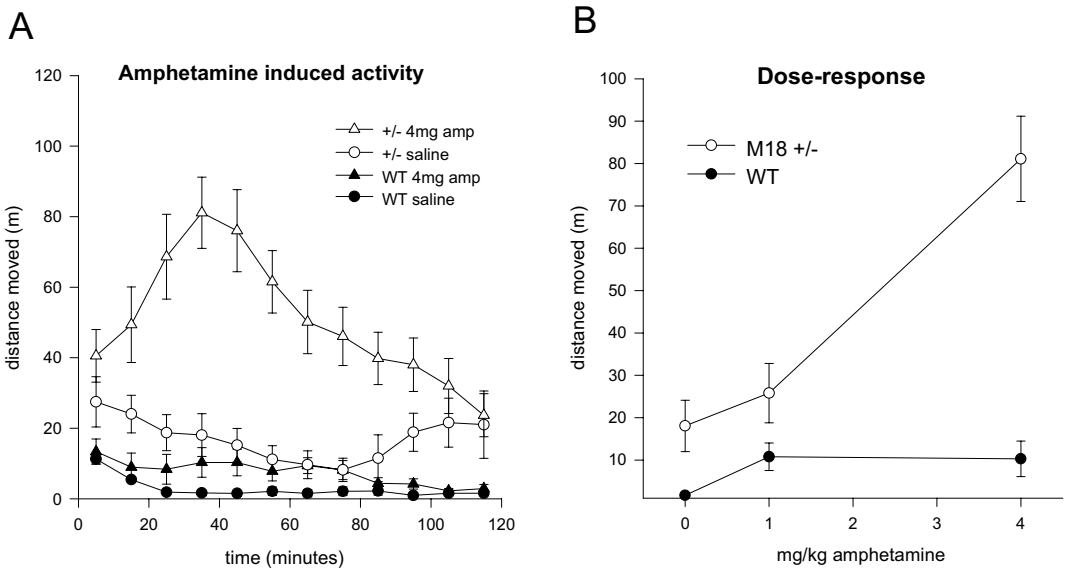

Figure 4. Response to amphetamine in wildtype (3 male and 3 female mice) and munc18-1 gene-dose mutants (3 male and 3 female mice). **A.** Response to 4 mg/kg amphetamine measured in 10min time bins. **B.** Dose response curve to 0, 1 and 4 mg/kg amphetamine. Y-axis shows moved distance in time-bin 4 (30-40min after injection). Significant effects: dose*genotype ($F_{1.74,13.9}$=16.6, p<0.001 (Huynh-Feldt corrected ε=0.87)), dose ($F_{1.74,13.9}$=22.7, p<0.001 (Huynh-Feldt corrected ε=0.87)) and genotype ($F_{1,8}$=26.5, p<0.005).

caused a strong increase of locomotor activity in gene-dose mutants and a relatively mild increase in wildtypes (Fig.4A). The dose-response relation (Fig.4B) shows that munc18-1 gene-dose mutants displayed a higher basal activity than wildtypes, which is moderately increased by 1 mg/kg AMPH but strongly by 4 mg/kg AMPH. Wildtypes showed relatively mild increases in activity when given 1 and 4 mg/kg AMPH. A significant interaction between dose and genotype was observed (p<0.001), indicating that munc18-1 gene-dose mutants respond stronger to AMPH than wildtypes. Thus, the hyperactive phenotype of munc18-1 gene-dose mutants is not alleviated by AMPH, but instead strongly increased, suggesting a dissimilarity with the mechanisms mediating hyperactivity in ADHD patients and certain animal models of hyperactivity (Solanto, 1998).

Discussion

Munc18-1 gene-dose mutants have a reduction of munc18-1 protein levels and display enhanced tetanic depression, absence of post-tetanic potentiation and a severe impairment of mossy fiber LTP. Despite all these changes no deficits in spatial learning and memory in the Morris water maze was detected. However, mutants did show severe deficits in the radial maze, a spatial working memory task. Furthermore, munc18-1 gene-dose mutants showed hyperactivity and responded strongly to d-amphetamine.

Munc18-1 gene-dose mutants performed normal in the Morris water maze. This task is widely used in learning and memory research and is sensitive to hippocampal lesions and disruptions of NMDA-receptor dependent LTP (Morris et al., 1982;Morris et al., 1986). Previously, other mutants with impairments in mossy fiber LTP were studied in this task. For instance, mGluR1 and AC1 null-mutants have impaired mossy fiber LTP and perform poorly in the water maze (Conquet et al., 1994;Wu et al., 1995;Villacres et al., 1998). However, mGluR1 mutants suffer from ataxia, which may have interfered with this task (Aiba et al., 1994;Conquet et al., 1994). Also, LTP in area CA1 is impaired in the AC1 mutant (Wu et al., 1995;Qi et al., 1996). Given the reported importance of CA1 LTP in spatial memory (Tsien et al., 1996), such impairments could also have caused deficits in this task. In contrast, rab3A null-mutants have disruption of both mossy fiber LTP and LTD and do not show impairments in spatial learning in the Morris maze (Castillo et al., 1997;Tzounopoulos et al., 1998)(chapter 5). Furthermore, mice that lack a catalytic subunit (Cβ1) or a regulatory subunit (RIβ) of PKA have no MF-LTP buts show normal Morris maze performance and normal contextual fear-conditioning (Huang et al., 1995). Our results are in agreement with the latter two studies and do not support a role for mossy fiber LTP in spatial learning and memory. Our results also show that post-tetanic potentiation (PTP) is not involved in spatial learning and memory in the Morris water maze. Previously, PTP has been implicated in spatial learning (Silva et al., 1996). αCaMKII gene-dose mutant mice show an enhancement of PTP and this mutant displays severe deficits in water maze learning (Silva et al., 1996). However, the results of the latter study appear to have been confounded by genetic background problems (Frankland et al., 2001). Thus, our results indicate that post-tetanic potentiation and mossy fiber LTP are not important for spatial learning in the Morris water maze.

Munc18-1 mice performed poorly in the eight-arm radial maze. Like the Morris water maze, performance in the radial maze is sensitive to hippocampal lesions (Olton, 1977;Olton et al., 1982). Furthermore, disruptions of NMDA-receptor dependent LTP impair performance in this working memory task (Danysz et al., 1988;Kawabe et al., 1998). However, impaired performance after NMDA-LTP blockade is only observed in a novel environment and not in a familiar one, provided that no lengthy within-trial intervals are imposed (Bolhuis and Reid, 1992;Shapiro and O'Connor, 1992). Thus, other mechanisms must be involved in the mediation of working memory *per se*. The munc18-1 mutant has impairments in tetanic depression, post-tetanic potentiation and mossy fiber LTP, which indicates that any of these different types of presynaptic plasticity may be involved in working memory. However, rab3A mutants lack mossy fiber LTP and show enhanced tetanic depression but perform normal in the eight-arm radial maze, suggesting that these types of plasticity are not involved in

working memory (Geppert et al., 1994)(chapter 5). As an alternative, post-tetanic potentiation may be involved in working memory (Churchland and Sejnowski, 1992). Like working memory, post-tetanic potentiation is short-lasting. Post-tetanic potentiation does not have properties of associativity like NMDA-receptor dependent LTP. If PTP is induced, it is likely to be induced in all synaptic connections of a neuron. However, this lack in specificity may not be necessary in working memory because the capacity of working memory is relatively low (Churchland and Sejnowski, 1992;Glassman, 1999). Furthermore, given the time frame of a few minutes before completing the radial maze task, it is hard to conceive of a mechanism for retention that does not contain some form of plasticity. Thus, post-tetanic potentiation may serve a function in working memory in the radial maze and this hypothesis is supported by our present findings.

A potential difficulty in interpreting our results is the fact that mutants showed increased activity in the radial maze. This was not observed in the Morris water maze, in which mutants performed normal. Hyperactivity is often associated with decreased attention and impulsive behavior and may result in poor cognitive performance (Solanto, 1998). Therefore, it may be suggested that the hyperactive phenotype of munc18-1 mutants interfered with radial maze performance but not with Morris water maze performance. On the other hand, variations in radial maze running speed in a variety of inbred strains do not correlate with radial maze performance, suggesting that higher locomotor activity does not affect radial maze performance to a large degree (Schwegler et al., 1990;Crusio et al., 1993;Ennaceur, 1994).

The hyperactive phenotype of munc18-1 gene-dose mutants was not alleviated by d-amphetamine, which has been reported to reduce activity in several animal models for hyperactivity (Solanto, 1998;Wilson, 2000;Zhuang et al., 2001). One of these models is the heterozygous coloboma mutant mouse. This mutant carries a chromosomal deletion of 1-2cM on chromosome 2, encompassing the gene for SNAP-25 (Wilson, 2000). This results in a reduction of SNAP-25 protein levels and also in reduced bodyweight and hyperactive locomotor behavior (Hess et al., 1996). The hyperactive phenotype of this mutant can be rescued by expression of a SNAP-25 transgene, which suggests a direct involvement of SNAP-25 (Steffensen et al., 1999). The function of SNAP-25 is related to munc18-1. Both proteins bind to syntaxin, and both are essential for neurotransmitter release (Montecucco and Schiavo, 1994;Sudhof, 1995;Verhage et al., 2000). In contrast to the munc18-1 gene-dose mutant, the coloboma mutant shows a reduction in locomotor activity after d-amphetamine injection, a response shared with ADHD patients (Hess et al., 1996;Solanto, 1998). Thus, although similar in many aspects, the coloboma and munc18-1 mutants differ markedly in amphetamine sensitivity, which suggest that different mechanisms are involved in the mediation of hyperactivity in these mutants.

In summary, mutant mice with impairments in tetanic depression, post-tetanic potentiation and mossy fiber LTP were analyzed in memory tasks. Spatial learning in the Morris water maze was not affected. Mutants showed poor performance in the radial maze, a working memory task. Furthermore, mutants showed hyperactive behavior that was not alleviated by d-amphetamine. Our results suggest a role for PTP in working memory, but interpretation is hampered by the additional hyperactive phenotype of the munc18-1 gene-dose mutant.