Chapter 2

A reduction of munc18-1 in mice leads to deficits in presynaptic plasticity.

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Abstract

The neuronal protein munc18-1 (also known as nSec1, Munc18-a and rbSec1) is essential for secretion of neurotransmitters. Deletion of munc18-1 results in a blockade of neurotransmitter secretion in mice. The effects of reduced levels of munc18-1 were studied in the central nervous system of the mouse. For this, munc18-1 gene-dose (or heterozygous) mutant mice were used. We show that gene-dose mutants have a reduction of munc18-1 protein levels throughout the brain, including the hippocampus. Neither the input-output relationship nor paired pulse facilitation was altered in hippocampal preparations of mutants, indicating that basal transmission and the ability to show enhancement of neurotransmitter release were not affected by reduced munc18-1 levels. However, repetitive stimulation at frequencies of 5, 10 and 20 Hz caused a greater depression of field potentials in mutants, suggesting that during high activity, neurotransmitter release was reduced in mutants. Post-tetanic potentiation is a form of short-term plasticity that lasts several minutes and appears mediated by increases of presynaptic calcium levels. Surprisingly, gene-dose munc18-1 mutants failed to show post-tetanic potentiation. Long-term potentiation was studied in area CA1 and at the mossy fibers of the hippocampus. Long-term potentiation in area CA1 was marginally reduced in gene-dose mutants, but mossy fiber long-term potentiation, which is expressed by an increase in neurotransmitter release, was virtually absent in mutants. In summary, a reduction of munc18-1 in gene-dose mutants leads to deficits in neurotransmitter release that is evident during and after episodes of high neuronal activity. Furthermore, our results suggest a novel and essential role for munc18-1 in two forms of presynaptic plasticity, post tetanic potentiation and mossy fiber LTP.

Introduction

Regulated secretion of neurotransmitter is the primary form of neuronal communication in the nervous system and is characterized by an extremely high speed and strict calcium dependence (Goda and Sudhof, 1997;Neher, 1998). Secretion is maintained by a specialized membrane trafficking cycle that includes assembly of secretory vesicles, transport to the active zone, vesicle docking, maturation and, finally, fusion with the plasma membrane (Sudhof, 1995;Lin and Scheller, 2000). A large number of proteins have been implicated in these steps, but the exact role of most of them remains unclear (Sudhof, 1995;Lin and Scheller, 2000). Fusion of neurotransmitter vesicles appears to involve the so-called SNAREs (soluble NSF attachment protein receptors) synaptobrevin/VAMP, syntaxin and SNAP25 (Chen and Scheller, 2001). Cleavage of any of these proteins by clostridial neurotoxins disrupts release of neurotransmitter (Montecucco and Schiavo, 1994;Jahn and Niemann, 1994) and together they seem to constitute the minimal requirement for membrane fusion (Weber et al., 1998). Syntaxin is associated with the plasma membrane and its availability appears to be regulated by munc18-1, which competes with synaptobrevin/VAMP for binding with syntaxin (Hata et al., 1993;Pevsner et al., 1994). Munc18-1 not only binds syntaxin but is also able to modulate syntaxin function by inducing conformational shifts (Dulubova et al., 1999;Misura et al., 2000;Jahn, 2000).

Munc18-1 (also known as nSec1, Munc18-a and rbSec1) is a mammalian member of the Sec1/Munc18-related proteins that appear to be involved in all eukaryotic membrane fusion reactions (Jahn, 2000). Munc18-1 is soluble and expressed throughout the brain (Garcia et al., 1994). Munc18-1 has been proposed as a negative but also as a positive regulator for neurotransmitter release (reviewed in (Jahn, 2000)). Null-mutant mice for munc18-1 have a disruption of neurotransmitter release in the brain and muscle-endplate (Verhage et al., 2000). Similarly, null-mutant flies for rop, the Drosophila ortholog of munc18, show a blockade of neurotransmitter secretion (Harrison et al., 1994). Furthermore, rop gene-dose (or heterozygous) flies show a reduction in neurotransmission (Wu et al., 1998). In contrast, a role as a negative regulator for neurotransmission was derived from overexpression of rop in Drosophila, which show reduced secretion of neurotransmitter (Schulze et al., 1994;Dresbach et al., 1998;Wu et al., 1998). Furthermore, rop overexpression results in decreased evoked responses during repetitive stimulation indicating that rop may be involved in short-term modulation of neurotransmitter release (Schulze et al., 1994).

Here, the effects of decreased levels of munc18-1 were studied in the central nervous system of the mouse. For this, gene-dose (or heterozygous) mutant mice for munc18-1 mutants were used. First, we established that heterozygosity leads to reduced protein levels in the brain. Second, the hippocampal slice preparation was used to study the effects of reduced munc18-1 levels on neurotransmission. Our results show that reduced munc18-1 levels results in stronger tetanic depression, absence of post-tetanic potentiation, and a severe impairment of long-term potentiation in the mossy fiber projection of the hippocampus.

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 were freely available. Gene-dose mutant and wildtype experimental animals were obtained by crossing heterozygote males with 129S3 females. Mice were genotyped by PCR. All experiments were approved by the Ethical Committee of the Utrecht University Medical Faculty.

Western blot

Brains of adult mice were dissected and the hippocampus, the cerebellum, the neocortex and brainstem were isolated. Proteins were separated and analysed by SDS-PAGE and Western blotting using 11% gels and semi-dry blotting onto nitro-cellulose membranes. Munc18-1 was detected with a monoclonal antibody (Transduction labs, Lexington, USA) and quantified with alkaline phosphatase conjugated secondary antibodies and enhanced chemifluorescence on a Molecular Dynamics Fluor Imager (Storm, Sunnyvale, USA). GDI was detected using similar procedures and the polyclonal antibody D633 (Verhage et al., 2000).

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 Schaffer collaterals. Field excitatory postsynaptic potentials (fEPSPs), determined from the linear part of the trace, were recorded in the stratum radiatum of area CA1 with glass microelectrodes (tip diameter approximately 2μm). fEPSPs were quantified by determining the slope of the trace. The input/output relation was determined with 5 stimulation intensities that were evenly spaced between intensities that induced threshold and maximum field potentials. 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. Paired-pulse facilitation was determined with a 50ms interval. Tetanic stimulation was at 5, 10 and 20Hz for 50 pulses. LTP was induced with 100Hz, 1s stimulation, as was PTP but with the addition of 50μM AP5 to the bath to block NMDA-receptors. Mossy fiber LTP was determined with a stimulation electrode at the afferent mossy fibers and recording electrode at the stratum radiatum of area CA3. Mossy fiber LTP was induced with a stimulation of 100Hz, 1s at half-maximum stimulation intensity.

Statistics

Results were analyzed using t-tests for independent samples, single-sample t-tests, univariate ANOVA and ANOVA for repeated measures. Huynh-Feldt correction was applied when the sphericity assumption of ANOVA for repeated measures was not met.

Results

Munc18 gene-dose mutants have reduced munc18-1 protein levels throughout the brain

Having only one copy of a gene may lead to lower expression levels of the protein. Unfortunately, there is no specific munc18-1 antibody suitable for immunocytochemistry. Therefore, we quantified munc18-1 levels using western blotting. In all brain areas tested, including the hippocampus, mutants showed a gene-dose dependent reduction in munc18-1 protein levels ($p<0.005$) (Table 1). GDI was a used as a general marker for neuronal proteins and was not reduced in munc18-1 gene-dose mutant mice (Table 1).

Table 1. Quantification of munc18-1 protein levels in brain tissue by western blot. Values (mean±sem) are measures of chemifluorescence intensities. Munc18-1 was detected using a monoclonal antibody (Transduction labs, Lexington, USA) and GDI was detected using a polyclonal antibody (D633). In all brain areas tested, including the hippocampus, mutants showed a gene-dose dependent reduction in munc18-1 protein levels. GDI was a used as a general marker for neuronal proteins and was not reduced in the mutant. Wildtypes: n=4, munc18-1 gene-dose mutants: n=4.

	Wildtype	**Munc18 +/-**	**Statistics**
Munc18-1			
Hippocampus	3.2 ± 1.0	0.71 ± 0.35	Genotype: $F_{1,6}=28.3$, $p<0.005$
Cortex	5.9 ± 0.85	2.9 ± 0.67	Area: $F_{3,18}=9.0$, $p<0.005$
Cerebellum	1.7 ± 0.63	0.56 ± 0.18	Area*Genotype: $F_{3,18}=0.62$, n.s.
Brainstem	4.3 ± 0.29	2.2 ± 0.75	
GDI			
Hippocampus	9.2 ± 3.2	6.4 ± 1.3	Genotype: $F_{1,6}=0.61$, n.s.
Cortex	7.8 ± 1.1	7.8 ± 0.9	Area: $F_{1.8,10.8}=9.4$, $p<0.01$ (Huynh-Feldt corrected $\varepsilon=0.60$)
Cerebellum	5.9 ± 0.4	5.8 ± 0.8	Area*Genotype: $F_{1.8,10.8}=0.49$, n.s. (Huynh-Feldt corrected $\varepsilon=0.60$)
Brainstem	13.0 ± 0.8	13.1 ± 0.6	

A reduction of Munc18-1 does not alter basal transmission and paired pulse facilitation

The hippocampal slice preparation was used to analyze the physiological consequences of reduced munc18-1 levels. First, the relationship between stimulus strength and field potential responses was tested. Five intensities were tested, evenly spaced between the threshold intensity and the minimal

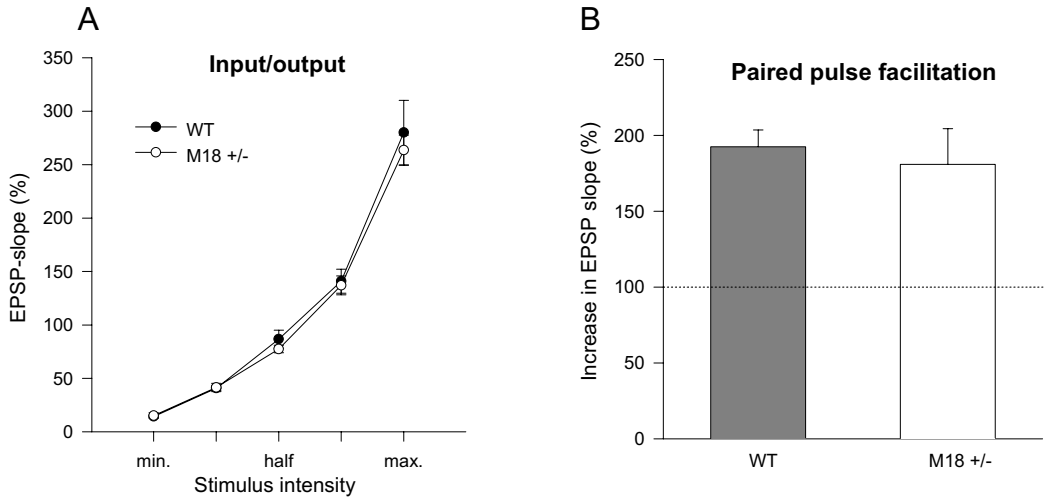

Figure 1. A. Relationship between stimulation intensity and field responses. The interval between threshold stimulation intensity and the minimal stimulation intensity that evoked a maximal response was divided in half to yield the halfmaximal stimulation intensity. A further division resulted in two further intermediate intensities. No differences were detected (genotype: $F_{1,18}$=0.58, n.s.; intensity*genotype: $F_{1.8,33.3}$=0.32, n.s., (Huynh-Feldt corrected ε=0.46)). Wildtype: 10 slices of 6 animals, munc18-1 gene-dose mutant: 10 slices of 5 animals. **B.** Paired-pulse facilitation. Halfmaximal stimulation intensity was used with a pulse interval of 50ms. No differences were detected (t(df=8)=0.45, n.s.). Wildtype: 5 slices of 5 animals, munc18-1 gene-dose mutants: 5 slices of 5 animals.

intensity that induced a maximum response. No differences were detected (Fig.1A), indicating that basal synaptic transmission was normal. Next, paired-pulse facilitation (PPF) was tested. PPF is a short-lasting enhancement of secretion believed to be caused by residual calcium (Zucker, 1999;Thomson, 2000a). PPF was tested with a 50ms interpulse interval. Both wildtypes and mutants showed PPF, and the strength of PPF was not different (Fig.1B). This shows that mutants are able to display a rapid enhancement of neurotransmitter release. Furthermore, this is an additional indication that basal transmission was not changed in mutants, because the strength of facilitation is believed to be correlated with initial release probability (Dobrunz and Stevens, 1997;Zucker, 1999;Thomson, 2000a).

A reduction of Munc18-1 results in enhanced tetanic depression

Tetanic stimulation was applied with frequencies of 5, 10 and 20Hz. Both wildtype and mutant slices showed depression of the response, but this was stronger in gene-dose mutants (Fig.2A, only 10Hz stimulation is shown). After 40-50 pulses, wildtypes showed a depression of 40%, while this was 58% in gene-dose mutants. The plateau phase that is reached after a number of pulses demonstrates the balance between replenishment and release. Mutants also showed a stronger depression at 5 and 20Hz. The relationship between the different stimulation frequencies and the amount of depression is summarized in Fig.2B. Mutants showed stronger depression than wildtypes with all stimulation frequencies ($p<0.001$). Furthermore, during 5Hz stimulation wildtypes showed only 10% depression, while this was already 40% in munc18-1 gene-dose mutants. This difference was significantly larger than the differences between mutants and wildtypes during 10 and 20Hz stimulation ($p<0.05$).

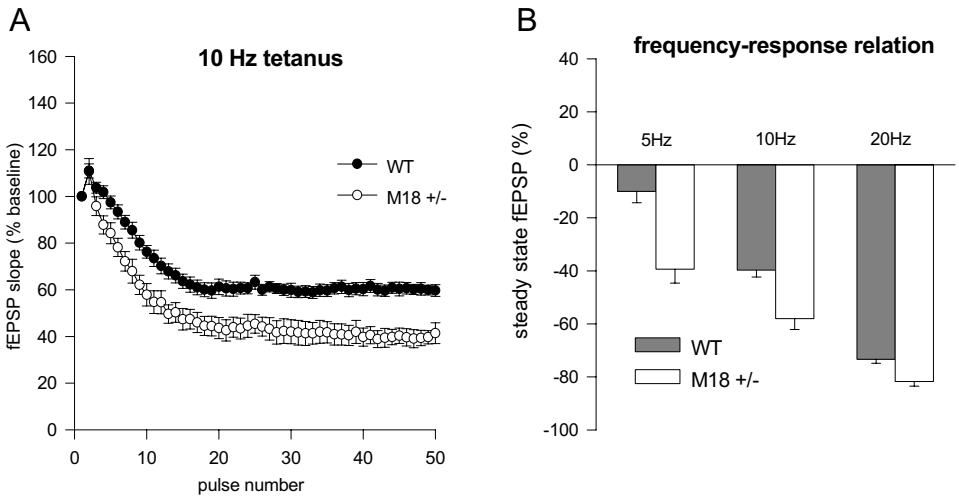

Figure 2. Tetanization experiments **A.** Field responses during 10Hz stimulation at halfmaximal intensity. Mutants showed stronger depression of field responses. Average of last 10 pulses (mean±sem): wildtype 60.3%±2.6, munc18-1 mutants 42.0%±4.1, t(df=13)=4.33, p<0.005. Wildtype 8 slices of 8 animals, mutants 7 slices of 7 animals. **B.** Summary of 5, 10 and 20Hz stimulation. Genotype*frequency: $F_{2,45}$=5.03, p<0.011; frequency: $F_{2,45}$=140.9, p<0.001; genotype: $F_{1,45}$=51.9, p<0.001. 5Hz: wildtype: 8 slices of 7 animals, mutants: 7 slices of 7 animals; 20Hz: wildtype: 12 slices of 10 animals, mutants: 9 slices of 8 animals.

Depression of neurotransmitter release is caused by vesicle depletion and the degree of depression is thought to reflect the refilling rate of the readily releasable pool (Dobrunz and Stevens, 1997;Stevens and Wesseling, 1998). Thus, a reduction of munc18-1 may have resulted in changes in the refilling rate of the readily releasable pool. However, tetanic stimulation will cause a rise in presynaptic calcium levels and induce facilitation, augmentation and post-tetanic potentiation (PTP) (Fisher et al.,

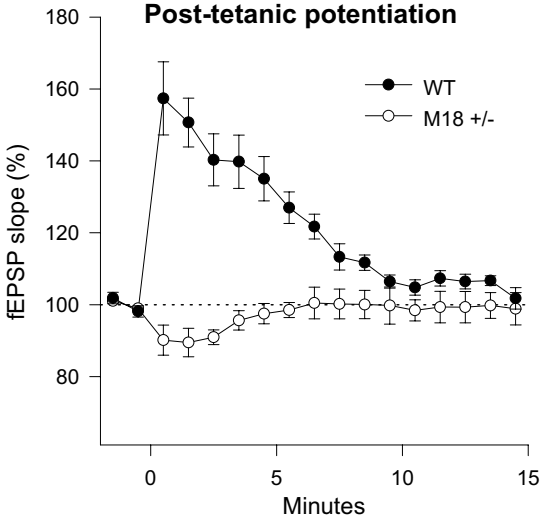

Figure 3 Post-tetanic potentiation. Slices were stimulated with a 100Hz tetanus at half-maximum stimulation intensity for 1s. AP5 (50µm) was added to the bath solution. Wildtypes showed potentiation of the response but munc18-1 mutants failed. Repeated measures ANOVA with 6 levels was used (t=1,3,5,7,9 and 11 minutes). Time*genotype: $F_{2.2,23.8}$=17.3, p<0.001, (Huynh-Feldt corrected ε=0.43). Wildtype: 7 slices of 6 animals, munc18-1 gene-dose mutant: 6 slices of 4 animals.

1997;Zucker, 1999). These enhancements of release may have decreased the degree of depression differentially in mutants and wildtypes. As was shown, facilitation was not changed in munc18 -1 gene-

dose mutants. When the readily releasable pool is emptied, refilling of the pool takes about 10 to 20s (Stevens and Wesseling, 1998). A measurement of augmentation, because of its short duration (~10s), will be contaminated by depletion of the readily releasable pool. Therefore, this hypothesis was tested by inducing PTP.

Reduced levels of munc18-1 levels abolish post-tetanic potentiation (PTP).

PTP was induced with a 100Hz tetanus that lasted 1 second. NMDA receptors were blocked by bath application of 50µM AP5. After induction, responses were measured every 20s giving ample time for refilling of the readily releasable pool. Wildtypes demonstrated PTP that lasted several minutes but munc18-1 gene-dose mutants failed and instead showed a transient decrease of the response ($p<0.001$) (Fig.3). Thus, a reduction of munc18-1 results in a blockade of post-tetanic potentiation.

A reduction of Munc18-1 causes minor deficits in CA1-LTP and severe deficits in mossy fiber LTP

Next, the effects of reduced munc18-1 levels on two different types of long-term plasticity were studied. Long-term potentiation (LTP) at the Schaffer/collateral-CA1 connection is NMDA-receptor dependent and believed to be expressed by post-synaptic changes (Malinow et al., 2000). LTP at this connection was induced with a 100Hz, 1s tetanus. Both heterozygotes and wildtypes showed LTP (Fig.4A), and it tended to be lower in heterozygotes, but the difference was not significant ($p=0.09$).

The second type of long-term plasticity that was studied was mossy fiber LTP. The mossy fibers are the axons from granule cells of the dentate gyrus and project to area CA3. Mossy fiber LTP is expressed by an enhancement of neurotransmitter release (Harris and Cotman, 1986;Staubli et al.,

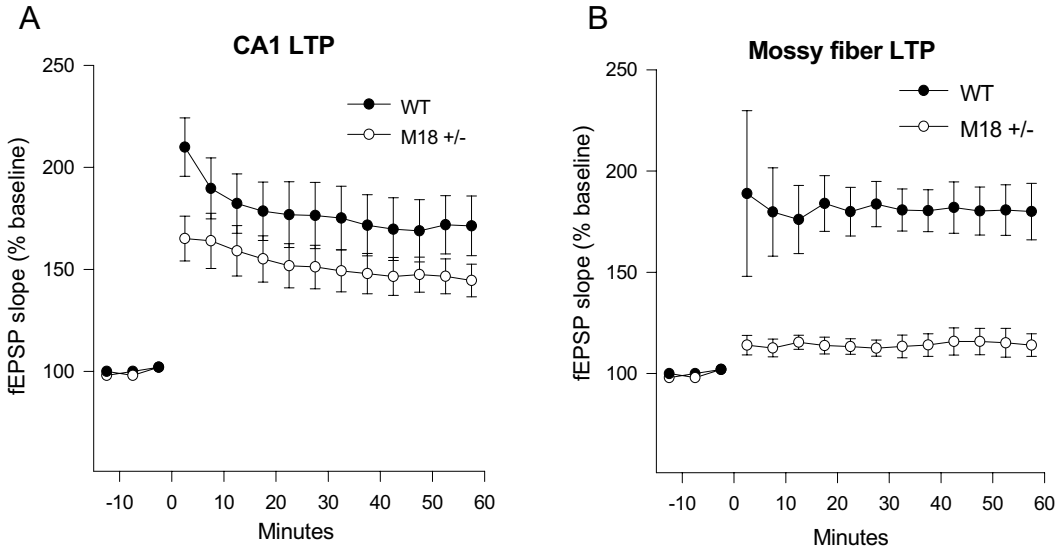

Figure 4 A. LTP in CA1. Changes in field EPSPs after tetanization (100Hz, 1s) of the Schaffer collaterals. Long lasting increases in field EPSPs are induced in wildtypes and munc18-1 gene-dose mutants (average responses 40-60 min after tetanization (mean±sem): wildtype 170%±15, mutants 146%±9, t(df=19)=1.38, p=0.092). Wildtype: 11 slices of 11 animals, munc18-1 gene-dose mutant: 10 slices of 9 animals. **B.** Mossy fiber LTP. Changes in field EPSPs after tetanization (100Hz, 1s) of the mossy fiber projection. Wildtype showed higher increases in field EPSPs than mutants (average responses 40-60 min after tetanization (mean±sem): wildtype 181%±13, munc18-1 gene-dose mutants 117%±6, t(df=12)=4.58, p<0.001). Munc18-1 mutants showed only little potentiation but it was more than baseline. One-sample t-test against 100% for munc18-1 mutants: t(df=6)=2.86, p<0.05. Wildtype: 7 slices of 7 animals, munc18-1 gene-dose mutant: 7 slices of 4 animals.

1990;Weisskopf and Nicoll, 1995). A 100Hz, 1s tetanus induced robust mossy fiber LTP in wildtypes (Fig.4B). Munc18-1 gene-dose mutants showed only a modest potentiation, which was higher than baseline ($p<0.05$), but different from wildtypes ($p<0.001$). Thus, a reduction of munc18-1 levels results in a severe impairment in a type of LTP that is expressed by enhancements of neurotransmitter release.

Discussion

The protein munc18-1 is essential for secretion of neurotransmitter from nerve terminals (Verhage et al., 2000). Presently, gene-dose mutants were analyzed at the biochemical and electrophysiological level. Mutants showed a gene-dose dependent reduction of munc18-1 protein levels throughout the brain. This reduction caused a number of electrophysiological changes. Although basal transmission and paired pulse facilitation appeared normal, mutants showed enhanced tetanic depression, absence of post-tetanic potentiation and a severe impairment in mossy fiber LTP. These results indicate that a reduction of munc18-1 results in a selective impairment in neurotransmitter secretion during and after periods of high neuronal activity.

Basal release and facilitation

The stimulus-response relationship appeared normal. This is in contrast to rop gene-dose mutant flies, which do show a decreased response to single stimuli (Wu et al., 1998). Perhaps, neurotransmitter release at the neuromuscular synapse in flies is more sensitive to decreased rop levels, as neurotransmitter release at central synapses in mice is to decreased munc18-1 levels. Alternatively, rop is involved in cellular membrane fusion reactions in non-neuronal cells (Harrison et al., 1994), and a decrease in rop levels may have led to general deficits in cell function. An additional measure for neurotransmitter release is paired pulse facilitation (PPF). PPF is a short-lasting enhancement of neurotransmitter release and, because of the strong correlation with release probability, is often used to measure changes in neurotransmitter release (Dobrunz and Stevens, 1997;Zucker, 1999;Thomson, 2000a). PPF was not changed in the munc18-1 gene-dose mutant, which is a further indication that basal release properties were not altered.

Post-tetanic potentiation

The absence of PTP in munc18-1 gene-dose mutants was unexpected and striking. PTP is dependent on moderate increases of presynaptic calcium and expressed by an enhancement of neurotransmitter release, which suggests the involvement of a high affinity calcium detector able to modulate neurotransmitter release (Kamiya and Zucker, 1994;Zucker, 1999). Little is known about the molecular mechanisms involved in PTP. Munc18-1 has been implicated in the translocation of peptide vesicles (Voets et al., 2001). Thus, translocation of new synaptic vesicles may be impaired in this mutant after high frequency stimulation of central synapses and cause a transient decrease of neurotransmitter release. However, given the normal timecourse of refilling (~20s) (Stevens and Wesseling, 1998), and the length of PTP in the present experiment (-6min), this does not appear to be able to explain the disruption of PTP. The deficit in refilling rate should be so large that tetanic stimulation at 5, 10 or

20Hz should have quickly resulted in complete extinction of secretion, and this was not observed. Therefore, our results suggest that decreases of munc18-1 disrupt some aspect of the expression of PTP and implicate munc18-1 in calcium-dependent presynaptic plasticity. Possible molecular mediators will be discussed below.

Tetanic depression

Munc18-1 gene-dose mutants showed an enhancement of tetanic depression. During tetanic stimulation a complicated interaction develops between depletion of the readily releasable pool of synaptic vesicles, enhancements of release by presynaptic calcium influx, and activation of inhibitory feedback. Any of these variables may have contributed to the changes in tetanic depression. For instance, the refilling rate of the readily releasable pool may have been altered. This hypothesis is supported by the fact that decreased munc18-1 causes impairments in the translocation of large peptide vesicles to the cell membrane (Voets et al., 2001). However, there are no indications for a similar role in trafficking of neurotransmitter vesicles in the central nervous system. For instance, null-mutants for munc18-1 have similar amounts of docked vesicles as wildtypes at birth (Verhage et al., 2000). Furthermore, impairments in recycling would probably be most evident at higher tetanic stimulation frequencies. However, the relationship between stimulation frequency and the amount of depression shows the opposite. The largest difference between wildtypes and mutants is during 5Hz stimulation and not at 10 or 20Hz (Fig2B). Another explanation for the differences in tetanic depression is increased inhibition by GABAergic interneurons. However, tetanic stimulation experiments were also performed at the neuromuscular junction by direct stimulation of motoraxons. In this preparation as well, mutants showed enhanced depression (Toonen et al., in prep). There is no inhibitory circuit in this preparation suggesting that enhanced depression in the hippocampal slice was not mediated by increases in the efficiency of inhibitory circuits. Finally, increased depression in munc18-1 mutants may have resulted from changes in the interaction between different types of presynaptic plasticity. Neurotransmitter release can be enhanced by facilitation, augmentation or post-tetanic potentiation (Fisher et al., 1997;Zucker, 1999;Thomson, 2000a). Differential expression of these types of presynaptic plasticity may have alleviated the amount of synaptic depression during tetanic stimulation differentially in wildtypes compared to mutants.

Relationship between facilitation, augmentation, post-tetanic potentiation and vesicle depletion

Facilitation is believed to be mediated by a local, high rise in calcium, while augmentation and PTP appear to be caused by a moderate, general rise in calcium (Fisher et al., 1997;Zucker, 1999). Facilitation was not changed in munc18-1 gene-dose mutants, indicating that the ability to show short-term enhancements of synaptic release is not affected by lowered levels of munc18-1. Augmentation and PTP are longer lasting types of presynaptic plasticity. There are indications that augmentation and PTP are mediated by similar molecular mechanisms. Both augmentation and PTP are directly related to presynaptic cytosolic calcium levels (Brain and Bennett, 1995), and both respond to sudden drops in calcium levels with similar time constants (Kamiya and Zucker, 1994). PTP is believed to be caused by calcium extrusion from mitochondria (Tang and Zucker, 1997), while augmentation seems to reflect a transient increase of cytoplasmatic calcium during moderate stimulation (Kamiya and Zucker, 1994). The different duration of augmentation (~10s) and PTP (~minutes) may just reflect different levels of

calcium buildup and time-course of extrusion (Fisher et al., 1997;Tang and Zucker, 1997). It is not likely that PTP was induced with the moderate stimulation frequencies that were applied during tetanic stimulation. However, as PTP was absent in munc18-1 gene-dose mutants, augmentation may also have been affected. Augmentation is readily induced with moderate tetanic stimulation, and a disruption of augmentation may explain the enhancement in tetanic depression in mutants. Clearly, further examinations are needed to elucidate this issue.

Mossy fiber LTP

Mossy fiber LTP is a form of LTP that is expressed presynaptically by an enhancement of neurotransmitter release (Yamamoto et al., 1980;Harris and Cotman, 1986;Staubli et al., 1990;Weisskopf and Nicoll, 1995). The mechanism of induction is not clear but appears to require postsynaptic calcium influx (Williams and Johnston, 1989;Yeckel et al., 1999). It may be argued that enhanced depression caused a decrease in neurotransmitter release during the induction of MF-LTP. However, MF-LTP was induced with short, high frequency stimulation (1s, 100Hz). This type of stimulation will probably result in secretion of all vesicles of the readily releasable, but appears too short to be influenced much by depression. The exact mechanisms of mossy fiber LTP are not known but synaptic vesicle proteins like rab3A and RIM have been implicated (Castillo et al., 1997;Castillo et al., 2000). Our results suggest that munc18-1 is involved in the induction or expression of mossy fiber LTP.

Molecular mechanisms

Our results show that a decrease in munc18-1 leads to deficits in the short- and long-term forms of presynaptic plasticity. The molecular mechanisms leading to enhanced neurotransmitter release are poorly understood. Our results implicate munc18-1 as an essential modulator of some of these processes. The role of munc18-1 may be mediated by munc18-1 interacting proteins such as syntaxin and Doc2 (Pevsner et al., 1994;Verhage et al., 1997;Duncan et al., 2000;Jahn, 2000). Doc2 contains high affinity calcium binding domains and has been implicated in the modulation of neurotransmitter release (Orita et al., 1995;Mochida et al., 1998;Kojima et al., 1996;Sakaguchi et al., 1999;Duncan et al., 2000). Syntaxin is essential for secretion of neurotransmitter (Bennett et al., 1992;Blasi et al., 1993). Furthermore, syntaxin is known to bind and inhibit calcium channels, and altered syntaxin function may cause changes in calcium kinetics (O'Connor et al., 1993;Leveque et al., 1994;Bezprozvanny et al., 1995;Stanley and Mirotznik, 1997;Sutton et al., 1999). The interaction between munc18-1 and syntaxin can be affected by PKC, which is able to phosphorylate munc18-1 in an activity dependent matter (Fujita et al., 1996;de Vries et al., 2000). Interestingly, activation of PKC results in an enhancement of neurotransmitter release and an increase in the size of the readily releasable pool (Carroll et al., 1998;Majewski and Iannazzo, 1998;Stevens and Sullivan, 1998;Yawo, 1999). Furthermore, PKC appears necessary for mossy fiber LTP (Son et al., 1996;Son and Carpenter, 1996). Perhaps the actions of PKC are mediated by munc18-1, and a decrease in munc18-1 levels may have altered these actions. Thus, a number of interactions exists by which munc18-1 may be involved in the modification of neurotransmitter release. However, further biochemical and electrophysiological characterization of these interactions is needed to elucidate the exact role of munc18-1.

In summary, munc18-1 gene-dose mutants were analyzed at the biochemical and electrophysiological level. Munc18-1 gene-dose mutant mice showed a gene-dose dependent reduction of munc18-1 protein levels throughout the brain which caused a number of electrophysiological changes. Mutants showed enhanced tetanic depression, absence of post-tetanic potentiation and a severe impairment in mossy fiber LTP, while basal transmission and paired pulse facilitation appeared normal. Our results suggest a novel function for munc18-1 in the induction and/or expression of short and long-term presynaptic plasticity.