A Role for Myotonic Dystrophy Protein Kinase in Synaptic Plasticity

PAUL E. SCHULZ,1,2,5 ADEKA D. MCINTOSH,1 MICHAEL R. KASTEN,1,2 BEREND WIERINGA,4 AND HENRY F. EPSTEIN1,2,3

1Department of Neurology, 2Division of Neuroscience, and 3Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030; 4Department of Cell Biology and Histology, University of Nijmegen, 6500 HB Nijmegen, The Netherlands; and The Houston VA Hospital Neurology Service

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Myotonic dystrophy (DM) is a multi-system disorder associated with an expanded triplet-repeat in the 3′-untranslated region of the gene for myotonic dystrophy protein kinase (DMPK), which may reduce DMPK expression. It is unclear how reduced DMPK expression might contribute to the symptoms of DM because the normal function of DMPK is not yet understood. Thus we investigated the function of DMPK to gain insight into how reduced DMPK expression might lead to cognitive dysfunction in DM. We recently demonstrated a role for DMPK in modifying the cytoskeleton, and remodeling of the cytoskeleton is thought to be important for cognitive function. Therefore we hypothesized that DMPK might normally contribute to synaptic plasticity and cognitive function via an effect on actin cytoskeletal rearrangements. To test for involvement of DMPK in synaptic plasticity, we utilized the DMPK null mouse. This mouse showed no changes in baseline synaptic transmission in hippocampal area CA1, nor any changes in long-term synaptic potentiation (LTP) measured 3 h after induction. There was a significant decrease, however, in the decremental potentiation with a duration of 30–180 min that accompanies LTP. These results suggest a role for DMPK in synaptic plasticity that could be relevant to the cognitive dysfunction associated with DM.

INTRODUCTION

Myotonic dystrophy (DM) is a multi-system disorder associated with an expanded triplet-repeat in the 3′-untranslated region of the gene for myotonic dystrophy protein kinase (DMPK). Myotonic dystrophy (DM) is associated with an expanded triplet-repeat in the 3′-untranslated region of the gene for myotonic dystrophy protein kinase (DMPK), which may reduce DMPK expression. It is unclear how reduced DMPK expression might contribute to the symptoms of DM because the normal function of DMPK is not yet understood. Thus we investigated the function of DMPK to gain insight into how reduced DMPK expression might lead to cognitive dysfunction in DM. We recently demonstrated a role for DMPK in modifying the cytoskeleton, and remodeling of the cytoskeleton is thought to be important for cognitive function. Therefore we hypothesized that DMPK might normally contribute to synaptic plasticity and cognitive function via an effect on actin cytoskeletal rearrangements. To test for involvement of DMPK in synaptic plasticity, we utilized the DMPK null mouse. This mouse showed no changes in baseline synaptic transmission in hippocampal area CA1, nor any changes in long-term synaptic potentiation (LTP) measured 3 h after induction. There was a significant decrease, however, in the decremental potentiation with a duration of 30–180 min that accompanies LTP. These results suggest a role for DMPK in synaptic plasticity that could be relevant to the cognitive dysfunction associated with DM.

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Several other lines of evidence also suggest that DMPK could have a role in synaptic plasticity. DMPK is a member of two networks already implicated in cytoskeletal dynamics and synaptic plasticity. The first network involves cell adhesion molecules such as integrins (Fig. 6: Adhesion Molecules), which are known to be important for short-term memory (Grotewiel et al. 1998). They activate members of the Rho family of small guanine nucleotide-binding proteins (GTPases) (Clark et al. 1998; Schwartz and Shattil 2000). This family includes Rac-1 (Fig. 6), Rho A, and Cdc42. These have been implicated in cell proliferation, differentiation, and extension via effects on the actin cytoskeleton. On the postsynaptic side of neuronal connections, Rac-1 and RhoA specifically regulate dendritic spine and branch numbers, respectively, and their complexity (Li et al. 2000; Nakayama et al. 2000). On the presynaptic side, the Rac GTPases are important for axonal growth, guidance, and branching (Hakeda-Suzuki et al. 2002; Ng et al. 2002). This network must be important for cognitive function since mutations in several members produce mental retardation (Allen et al. 1998; Billuart et al. 1998). We recently showed that Rac-1 GTPase also activates DMPK (Fig. 6) (Shimizu et al. 2000). Since the Rho/Rac-1 network is so important for cytoskeletal dynamics and cognitive function, the finding that they activate DMPK raises the possibility that DMPK is also involved in those functions.

DMPK is also a member of another network that is important for synaptic plasticity. Extracellular ligands (Humoral factors and neurotransmitters; Fig. 6) can activate receptor–tyrosine kinase complexes that activate the small GTPase Ras. Ras can activate Raf-1. This Ras-Raf system is linked downstream to mitogen-activated protein kinase (MAPK; Fig. 6) (Frost et al. 1996), which is required for hippocampal LTP induction (English and Sweatt 1997) and memory (Atkins et al. 1998). We recently demonstrated that Raf-1 also activates DMPK in vitro (Fig. 6) (Shimizu et al. 2000). This again places DMPK in a network that is critical for synaptic plasticity.

DMPK is activated, then, by two systems that are important for actin cytoskeletal dynamics and therefore for synaptic plasticity. Adhesion molecules activate one system while neurotransmitters and humoral factors stimulate the other. DMPK, in turn, can activate cytoskeletal actomyosin. Thus we hypothesized that DMPK could have a role in the cross-talk between the adhesion-dependent and chemically stimulated transduction systems as they impact synaptic function and plasticity (Shimizu et al. 2000). We tested for such a role of DMPK in synaptic function by examining synaptic transmission and plasticity in the DMPK knockout mouse, and we report the loss of a unique phase of synaptic potentiation.

METHODS

DMPK null mice

Gene knockout models have been very useful for dissecting the mechanisms underlying learning and memory in multiple systems (Steele et al. 1998) including Drosophila (e.g., volado and dunce) and mice [e.g., glutamate receptor subunit 2, the cAMP cascade, Ras guanine nucleotide-releasing factor, and CA1 N-methyl-D-aspartate (NMDA) receptors]. To test the hypothesis that DMPK has a role in synaptic plasticity, we examined hippocampal synaptic plasticity in the DMPK knockout mouse.

Dr. Wieringa’s laboratory at the University of Nijmegen (Nijmegen, The Netherlands) developed the DMPK null mice. Jansen et al. (1996) knocked out exons one through eight of the Dmpk gene in a C57BL/6 line of mice. They characterized this mouse line as null for DMPK at both the RNA and the protein levels. DMPK RNA was reduced 50% in heterozygotes and was absent in homozygotes by Northern analysis with Dmpk cDNA. Similarly, Western blots using three different polyclonal antibodies to the DMPK protein demonstrated a reduction of DMPK to undetectable levels in brain.

We crossed the DMPK null mice with normal C57 mice to produce heterozygotes and then mated brother-sister heterozygotes to produce litter with wild-type (+/+), heterozygous (+/−), and homozygous null (−/−) genotypes. Genotypes were determined by preparing DNA from amputated tails, and Southern blotting of BamH I digests. The wild-type and knockout DMPK haplotypes were detected as 4.5 and 6.5 kb BamH I restriction fragments, respectively, using a 500-bp fragment of DMPK cDNA as a probe. There was adherence to the American Physiology Society’s policies regarding the use and care of animals.

Hippocampal slice preparation

DMPK protein localizes to the dentate gyrus and the CA1 region of hippocampus (Jansen et al. 1996; Whiting et al. 1995). Hippocampal slices were prepared as follows: 10- to 12-wk-old mice (C57BL/6J, 16–30 g) were anesthetized with a combination of ketamine (42.8 mg/ml), xylazine (8.6 mg/ml), and acepromazine (1.4 mg/ml) at 0.5–0.7 ml/kg ip. When the animal did not respond to tail pinch, a cannula was inserted in their left ventricle, which was perfused with iced saline containing the following (in mM): 125.00 NaCl, 2.50 KCl, 1.25 NaH2PO4, 25.00 NaHCO3, 0.50 CaCl2, 7.00 MgCl2, 25.00 dextrose, and 1.00 ascorbate. A cut was made in their right atrium to allow extravasation. When the feet pads and nose were pale, animals were decapitated; their hippocampi were harvested, and 400-μm-thick transverse slices were made using a Vibratome (Technical Products International). Hippocampal slices were cut in iced saline containing the following (in mM): 110 sucrose, 60.00 NaCl, 3.00 KCl, 1.25 NaH2PO4, 28.00 NaHCO3, 0.50 CaCl2, 7.00 MgCl2, 5.00 Dextrose, and 0.60 ascorbate. Added to the cutting saline to block NMDA currents was 100 μM D,L-aminoephosphonovaleric acid (APV). A surgical cut between CA3 and CA1 prevented epileptiform activity.

Electrophysiologic recordings

Slices were transferred to a Haas-type (Haas et al. 1979) interface-recording chamber (Medical Systems) at 32.5 ± 0.1°C. The recording saline contained the following (in mM): 124 NaCl, 4.4 KCl, 26 NaHCO3, 1 NaH2PO4, 1.3 MgCl2, 10 dextrose, 2.5 CaCl2, and 4 μM picrotoxin. All salines were gassed with 95% O2–5% CO2. Microelectrodes were pulled from 1.5 mm OD glass tubing using a Flaming/Brown micropipette puller (Sutter Instrument). Extracellular electrodes were filled with artificial cerebrospinal fluid (ACSF; resistances = 1 to 3 MΩ). Stimulation was for 50 ms at 20-s intervals via bipolar, Teflon-coated platinum electrodes. Extracellular recordings were made in area CA1. This technique is particularly well suited to the multi-hour recordings necessary for the results reported. A single- or two-pulse stimulation intensity that initially yielded a 1-mV population excitatory postsynaptic potential (pEPSP) was used throughout each experiment. At baseline, synaptic integrity was tested in several ways. This included the input-output curve, which compares the ratios of stimulus strength to pEPSP slope.

Slices received high-frequency stimulation (HFS) at 100 Hz for 1 s, times four, at 20-s intervals. For the HFS, the stimulus intensity was increased one-third of the way from the baseline intensity to the intensity that yielded the maximal pEPSP slope. Data were acquired and analyzed on intel- (Bifrost, Houston, TX) and Motorola-based (NeXT) computers using the NeXtStep operating system, which was running the DAM program written by Costa Colbert. pEPSP slopes were determined by linear regression over the maximum initial slope.
points. To avoid bias, we analyzed the same time points throughout the experiment. Standard statistical tests were used as noted for each figure (Zar 1984).

Experimentalists were unaware of animal genotypes and no external features suggested the genotype, thereby keeping experimentalists blinded. Each litter was tested neurophysiologically and then was tested for genotype by polymerase chain reaction analysis of the animal’s tails. All animals tested were littermates of the same age and genetic background, except for the control group labeled "wild non-littermates." Only one slice was used from each animal.

RESULTS

Normal baseline synaptic transmission

To assess synaptic transmission in the DMPK null mice, we used three neurophysiologic measures of synaptic integrity: input-output curves, trace morphology, and paired-pulse facilitation (PPF).

Input-output curves quantify the ratio of stimulus intensity-to-pEPSP slope. This formally tests whether a range of stimulus intensities produce a normal range of pEPSPs. Stimulus intensities and pEPSP slopes were averaged to produce the input-output curves of Fig. 1A, and the curves were similar (n = 6 −/−, 10 +/+−, and 17 +/+). Normalizing the curves to the maximum stimulus and pEPSP slope values also produced similar plots for each group (Fig. 1B). The similarity of input-output curves across groups suggests normal synaptic physiology in the −/− group. The morphology of the pEPSPs in −/− animals was also normal (Fig. 1C). Increasing stimulus intensity produced a family of normal appearing pEPSPs.

PPF is an increase in a second pEPSP when it follows shortly after a first. Presynaptic mechanisms contribute to PPF expression (Foster and McNaughton 1991; Katz and Miledi 1968). PPF measurement is useful because changes in presynaptic function lead to changes in PPF (Schulz et al. 1994, 1995, 1997). Conversely, a lack of change in PPF suggests normal presynaptic function. Thus we assessed presynaptic function by testing PPF.

As shown in Fig. 2A, the morphologies of paired-pulses were similar in +/+−, +/+−, and −/− slices. Formal assessment of PPF, defined as the ratio of the initial slope of the second pEPSP to the initial slope of the first pEPSP, demonstrates that PPF was the same across groups (Fig. 2B). It was 46.9 ± 2.1%.
Values are means ± SE except for significance. There were no statistically significant differences in PPF between wild, heterozygous, and homozygous null animals (n = 29, 10, and 7). The probability of developing LTP and the magnitude of LTP induced in the three groups also did not differ. There was a decrease, however, in the magnitude of decremental LTP in the myotonic dystrophy protein kinase null slices whether one examined only slices that showed LTP (second row from the bottom) or all slices given high-frequency stimulation (bottom row). PPF, paired-pulse facilitation; LTP, long-term synaptic potentiation.

Comparing LTP across groups

In these example slices, LTP was elicited by first obtaining stable baseline pEPSPs and then giving HFS (Fig. 3A, at the arrows). In the literature, LTP is defined as an increase in the pEPSP at various times after HFS, including 2 min (Isaac et al. 1995; Liao et al. 1995), 30 min (Oliet et al. 1997), and 40–45 min (Misner et al. 2001; Xu et al. 2000). We initially used a
later time point, i.e., 60 min after HFS. In this example, HFS to a +/− slice produced >100% LTP at 1 h after HFS. In contrast, HFS to a −/− slice produced only 35% LTP. To the right, we compare baseline traces to those obtained 20 (top) and 60 (bottom) min after HFS for +/− (left) and −/− (right) slices (scale bars = 10 ms and 1 mV). While we assessed pEPSP slope in these experiments, it is also obvious that there is a smaller increase in amplitude in the −/− versus the +/− slice at time “c.”

Decremental phases of potentiation may accompany the expression of LTP. One form, with a duration <6–15 min, is referred to as short-term potentiation (STP) (Huang and Kandel 1994; Schulz and Fitzgibbons 1997). This potentiation is usually absent after multiple HFS such as those used here (Schulz and Fitzgibbons 1997). Longer duration, decremental phases of LTP are referred to as E-LTP, I-LTP, and protein synthesis-independent potentiation (Abel et al. 1997; Frey et al. 1993; Winder et al. 1998). They have durations of 40 min to several hours. To be certain that we were studying LTP and not a decremental phase of potentiation, we followed slices until the pEPSP slopes were constant for >30 min.

One typically observes the three longer lasting decremental potentiations after HFS in the presence of inhibitors of transcription and translation (Frey and Morris 1997) or after single HFS (Huang et al. 2000), and thus we did not expect to observe long-lasting decremental potentiation in the absence of such inhibitors or after giving multiple HFS. It was surprising to find, then, that decremental potentiation with a duration of 2–5 h always accompanied LTP expression. This produced the unexpected result shown in Fig. 3B. This is a plot of the same slices shown in Fig. 3A with an additional 2 h of recording. Instead of 100 and 35% LTP, it now appears that there was similar LTP in each slice (Fig. 3B). Traces from the +/− and −/− slice also indicate that LTP magnitudes were similar when measured late after HFS (right). This figure demonstrates that even defining LTP as the potentiation present 60 min after HFS does not rule out the presence of decremental potentiation. It also demonstrates that the majority of potentiation present shortly after HFS may be decremental as opposed to sustained.

The decremental component of LTP could result from drop-out of axonal fibers during the experiments. To rule this out, we monitored the fiber volley during each experiment. An example is shown in Fig. 3C. Here, the fiber volleys from the experiments shown in Fig. 3B are plotted over time. The fiber volley was defined as the area under the curve marked by vertical lines in the traces of Fig. 3C (* marks the stimulus blanking). The plots in Fig. 3C indicate stability of the fiber volley for the 3 h of the recordings. The traces to the right are the average of all traces before and after high-frequency stimulation: the upper traces are the heterozygous slice and the lower traces are the DMPK null slice. The overlap of fiber volleys in these traces also indicates that fiber volley did not change during these experiments. Thus changes in fiber volley do not appear to account for the decrease in decremental LTP in the DMPK null slice of Fig. 3B.

Knockout mice have normal LTP, but decreased decremental potentiation

All slices showed long duration, decremental potentiation. When this decremental potentiation fully decayed, some slices
showed LTP, but others had returned to baseline. LTP was defined as the potentiation present at the end of each experiment when eEPSP slopes were constant for ≥30 min. This usually occurred ≥2.5 h after HFS. We compared the magnitudes of both decremental and sustained potentiation across groups of slices.

Figure 4A compares the time courses of synaptic potentiation for the slices that showed LTP at the late time point after HFS. At 2.5 h after HFS, sustained potentiation was equivalent between homozygous (●), 141.8 ± 13.6% (100% = baseline), n = 5) and wild slices (◇, 153.0 ± 14.7%, n = 7, P > 0.05). This potentiation is smaller than is sometimes reported at earlier times after HFS since we waited until the decremental phases had decayed. The probability of developing LTP was also similar for all three groups (Table 1; 7/16 for +/+), 3/7 for +/−, and 5/7 for −/−).

Despite similar LTP amplitudes, decremental potentiation differed significantly between groups. Subtracting sustained potentiation from each slice in Fig. 4A and plotting the resulting decremental potentiation on an expanded y-axis produced Fig. 4B. We subtracted 141.8% from each homozygous point in Fig. 4A and 153.0% from each wild point. There was a reduction in decremental potentiation at 5 to 20 min after HFS from 51.9 ± 18.4% in +/+ slices (◇, n = 7) to 18.2 ± 7.0% in −/− slices (●, n = 5). This 64.9% reduction was significant (Table 1, P < 0.05).

Figure 4C shows the result of averaging all slices from the wild and knockout groups, including those that eventually showed LTP and those that showed only decremental potentiation (i.e., those that returned to baseline). The average sustained potentiation was identical between these two groups of slices (125.9 ± 14.3% in −/− slices vs. 123.6 ± 9.9% in +/+ slices, P > 0.05). Subtracting sustained potentiation from the points in Fig. 4C and expanding the y-axis (as was done for Fig. 4B) produced Fig. 4D. This again demonstrates a significant decrease in decremental potentiation from 77.7 ± 11.7% (n = 15) in +/+ slices to 24.3 ± 6.5% in −/− slices (n = 7, 68.7% reduction, Table 1, P = 0.0003).

Figure 4, E and F, shows the result of averaging all slices from the heterozygous and homozygous null mice, including those that eventually showed sustained potentiation and those that showed only decremental potentiation, i.e., that returned to baseline. The two groups showed identical sustained potentiation (Fig. 4E, 125.9 ± 14.3% in −/− slices, n = 7, vs. 112.6 ± 9.1% in +/+ slices, n = 7, ◇). There was also identical potentiation among slices selected for showing sustained potentiation (i.e., >20% potentiation at 2.5 h after HFS), which was 141.8 ± 13.6% in −/− slices (●) and 134.8 ± 7.3% in +/+ slices (◇, P > 0.05, data not shown). There was a significant reduction, however, in decremental potentiation from 62.6 ± 12.2% in +/+ slices (Fig. 4F, n = 7) to 24.3 ± 6.5% in −/− slices (Table 1, n = 7, 62.1% reduction, P = 0.007).

Finally, we compared the amplitude of decremental potentiation between wild (◇ in Fig. 4C) and heterozygous slices (△ in Fig. 4E). There was 62.6 ± 12.2% in the +/+ slices (n = 7) versus 77.7 ± 11.7% in the +/+ slices (n = 15), which is not significantly different (Table 1, P > 0.05).

The data indicate that if one defines LTP as the potentiation present between 2 and 60 min after HFS, then there is more LTP in the wild and heterozygous slices than the knockout slices. If, however, one defines LTP as sustained potentiation, which is probably more relevant to sustained memory, then LTP across groups is equivalent and decremental potentiation is decreased approximately 70% in the −/− group.

Lack of a relationship between the magnitudes of decremental and sustained potentiation

The data thus far indicate that baseline synaptic transmission and sustained potentiation are similar between the three groups of slices, but decremental potentiation differs. The relationship between the magnitudes of the sustained and decremental phases of LTP across the three groups was explored further. We hypothesized that if decremental and sustained potentiation were expressed through the same mechanisms, then the increased expression of one would decrease the expression of the other. Thus a plot of one versus the other would produce a significant inverse relationship.

Figure 5 shows the results of plotting the magnitude of sustained (x-axis) versus decremental (y-axis) potentiation for each individual slice from each group. Contrary to expectations, there was no significant inverse relationship between sustained and decremental potentiation for any subset of slices. This included the +/+ littermates and nonlittermates (Fig. 5A, ○ and △, n = 16, r = 0.03, P > 0.05), heterozygotes (Fig. 5B, ○, n = 7, r = 0.12, P > 0.05), homozygotes (Fig. 5C, ●, n = 7, r = 0.57, P > 0.05), or a combination of all groups (Fig. 5D, n = 30, r = 0.01, P > 0.05).

Inspection of these plots also confirms that there is less decremental potentiation in the −/− slices: the decremental potentiation plotted on the y-axis in Fig. 6, C and D, (−/− slices, ●) is much less than the potentiation plotted on the y-axis in 6A (wild), B (+/+ slices), or D (all slices).

![Figure 5](http://jn.physiology.org/). Absence of a significant relationship between decremental and sustained LTP magnitudes. There was no relationship between decremental and sustained LTP in +/+ slices (A), +/− slices (B), −/− slices (C), or a combination of all groups (D). It is also obvious that there was less decremental potentiation (plotted on the y-axis) in the −/− group (C) vs. the other 2 groups (A, B, and D). This lack of a relationship between the magnitude of decremental and sustained potentiation suggests that differing mechanisms underlie the expression of these 2 phases of LTP.
sustained potentiation (Kim and Lisman 1999) and could conceivably contribute to the expression of decremental potentiation as well. A recent finding of ours may be relevant to the underlying mechanism. DMPK phosphorylates the myosin-binding subunit of myosin phosphatase (Muranyi et al. 2001), which inhibits its dephosphorylation of myosin. The phosphorylation state of myosin affects the assembly and function of the actin cytoskeleton (Schoenwaelder and Burridge 1999). The loss of DMPK in our experiments, then, may have led to a reduction of actin-dependent changes in the cytoskeleton and hence a decrease in decremental LTP. Figure 6 illustrates this model for the involvement of DMPK in decremental synaptic potentiation. Several connections in this cascade have yet to be demonstrated experimentally in hippocampus. Nonetheless, our working hypothesis is that reduced DMPK expression in DM could decrease the phosphorylation state of myosin and hence interfere with any myosin-mediated cytoskeletal contributions to decremental plasticity.

**DMPK null mice exhibit several features of DM**

Several disease mechanisms are proposed to account for the symptoms of DM, including decreased DMPK production (haploinsufficiency), decreased expression of neighboring genes (Gennarelli et al. 1999; Klesert et al. 2000; Korade-Mirnics et al. 1999), and a toxic gain-of-function due to altered RNA processing (Amack et al. 1999; Mankodi et al. 2000; Miller et al. 2000). The DMPK null mouse is an important test of whether decreased DMPK production may contribute to the symptoms of DM.

Several experimental results suggest that haploinsufficiency can account for some symptoms of DM. First, there is evidence for decreased DMPK expression in DM (Carango et al. 1993; Fu et al. 1993; Hofmann-Radvanyi et al. 1993; Ueda et al. 1999). Second, there is an inverse relationship between DMPK dosage and the cardiac conduction defect frequency in DMPK null mice (Berul et al. 1999). Those arrhythmias also increase with age, mimicking another feature of DM. Third, DMPK null mice have at least some myopathic features of DM (Jansen et al. 1996; Reddy et al. 1996). Fourth, muscle fibers and/or cultured skeletal muscle cells of DM patients exhibit a decreased resting membrane potential and increased basal cytosolic Na\(^+\) and Ca\(^{2+}\) concentration (Benders et al. 1996). Muscle from DMPK null mice also shows higher resting intracellular calcium than wild-type muscle (Benders et al. 1997). Finally, this study demonstrates changes in synaptic plasticity that may be relevant to the cognitive dysfunction associated with DM.

A toxic gain-of-function also may account for some symptoms of DM. Transgenic mice expressing an expanded CUG repeat in muscle, for example, show classical myotonia and the morphology of DM. They do not, however, appear to have weakness of skeletal muscle (Mankodi et al. 2000). Expanded repeats may also not account for mental retardation in DM since the DM-related human disorder DM2 has a repeat expansion in a transcribed but noncoding sequence (Liquori et al. 2001), but it is not associated with mental retardation. Knockout of the neighboring Six5 (DMAHP) gene produces cataracts, a characteristic finding in DM, but no other features of DM are evident (Sarkar et al. 2000; Klesert et al. 2000). Thus each of the three proposed mechanisms for DM may account for some but not all symptoms of DM, thereby supporting the hypothesis.
that a combination of mechanisms may be necessary to account for all symptoms.

**What form of decremental potentiation did DMPK knockout reduce?**

The decremental potentiation reduced by DMPK knockout has a time course of 30 min to several hours (Figs. 4 and 5). This time course is similar to that of E-LTP, which has a duration of 40 min (Winder et al. 1998) to 3 h (Frey et al. 1993). There appear to be several differences, however, between E-LTP and the decremental potentiation under study here. E-LTP is typically induced by “a single high-frequency train of stimuli” (Huang et al. 2000) or is observed “in the presence of inhibitors of transcription and translation” (Frey and Morris 1997). The potentiation observed here, in contrast, was induced by four high-frequency trains in the absence of inhibitors.

Longer HFS are also suggested to convert E-LTP to L-LTP (Frey et al. 1993). This hypothesis predicts that there would be an inverse relationship between the magnitudes of E-LTP and L-LTP. This was not the case here, however, where there was no relationship between the magnitudes of decremental and the sustained potentiation (Fig. 5). There appears, then, to be several differences between E-LTP and the decremental potentiation under study here.

Regardless of the type of decremental potentiation under study here, this appears to be the first example of a gene knockout reducing decremental potentiation while leaving sustained potentiation intact. The fact that decremental potentiation could be reduced independently of sustained potentiation suggests that the two potentiations involve independent mechanisms.

**Insights into the mechanisms of mental retardation**

Our understanding of the physiologic basis for mental retardation is limited. Including the DMPK knockout mouse reported here, there appear to be six mouse models of a disorder associated with mental retardation. Two animal models have normal LTP: our DMPK knockout and fetal alcohol syndrome (Bellinger et al. 1999). Two models show decreased LTP: Down syndrome (Siarey et al. 1997) and Angelman syndrome (Bellinger et al. 1999). Two models show decreased LTP: our DMPK knockout and fetal alcohol syndrome (Siarey et al. 1997) and Angelman syndrome (Bellinger et al. 1999). Two models show decreased LTP: our DMPK knockout and fetal alcohol syndrome (Siarey et al. 1997) and Angelman syndrome (Bellinger et al. 1999). Two models show decreased LTP: our DMPK knockout and fetal alcohol syndrome (Siarey et al. 1997) and Angelman syndrome (Bellinger et al. 1999). Two models show decreased LTP: our DMPK knockout and fetal alcohol syndrome (Siarey et al. 1997) and Angelman syndrome (Bellinger et al. 1999). Two models show decreased LTP: our DMPK knockout and fetal alcohol syndrome (Siarey et al. 1997) and Angelman syndrome (Bellinger et al. 1999).

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