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The Involvement of PTEN in Sleep Deprivation-Induced Memory Impairment in Rats

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ABSTRACT

Although the underlying mechanism is not elucidated, it has been postulated repeatedly that deprivation of sleep, particularly rapid eye movement (REM) sleep, affects learning. Here we report that memory for newly acquired information is impaired after a specific period of REM sleep deprivation (REMD). Memory retrieval-induced phosphorylation of protein kinases in the rat amygdala is abrogated by REMD that is associated with an increase in the expression of a dual protein/lipid phosphatase PTEN. REMD given before training is without effect, suggesting the lack of effect on the acquisition of memory. Intra-amygdala administration of antisense but not sense or scrambled oligonucleotides for PTEN prevents REMD-induced decrease in protein phosphorylation and impairment of fear memory. Thus, REMD interferes with the process of memory retention via the activation of PTEN.

On the basis of the patterns of brain electrical activity measured with electroencephalogram, sleep is broadly divided into REM and non-REM sleep (Carskadon and Dement, 2000). REM is characterized by low-amplitude, relatively fast rhythms on electroencephalogram recording, rapid eye movement, and decreased muscle tone (Maquet, 2001). There is a long history of research suggesting that sleep, REM sleep in particular, is necessary for the processes of memory consolidation (Maquet, 2001; Stickgold et al., 2001). This idea implies that patterns of neuronal activity present during waking are reactivated during subsequent REM sleep that converts the labile neural changes into a stable form (Graves et al., 2001; Benington and Frank, 2003). Consistent with this idea, increase in REM sleep has been observed after successful task acquisition (Lucero, 1970; Smith, 1996). In addition, REM sleep deprivation at certain post-training times impairs memory for recently acquired tasks (Fishbein, 1971; Smith and Rose, 1996; Graves et al., 2003). Furthermore, long-term potentiation (LTP) of excitatory synaptic transmission in the hippocampus, a candidate mechanism for learning and memory, is reduced by sleep deprivation (Campbell et al., 2002; Davis et al., 2003). On the other hand, the induction of LTP during waking leads to an up-regulation of *zif*-268 gene expression during subsequent REM sleep (Ribeiro et al., 2002). However, there are also different opinions arguing against the evidence of reactivation processes relating to memory consolidation that take place during sleep (Vertes and Eastman, 2000; Siegel, 2001).

PTEN is a tumor suppressor gene located in the chromosome 10q23 region, a place frequently targeted for genetic loss in tumors. Indeed, heterozygous disruption of the *PTEN* gene in knockout mice results in the spontaneous development of tumors (Stambolic et al., 1998). With a function similar to that of the *PTEN* gene, PTEN protein mutation is associated with several cancers. We have demonstrated recently that acquisition of fear memory was associated with an activation of phosphatidylinositol 3-kinase (PI-3 kinase) and its downstream target Akt in the rat amygdala (Lin et al., 2001). PI-3 kinase and Akt were also activated in response to LTP-inducing tetanic stimulation. In parallel, PI-3 kinase inhibitors interfered with tetanic stimulation-induced LTP as well as long-term fear-memory formation. PTEN is a dual protein/lipid phosphatase (Maehama and Dixon, 1998) that dephosphorylates the 3′ position of PI-3 kinase product phosphatidylinositol 3,4,5-triphosphate and consequently down-regulates PI-3 kinase pathway. Therefore, it is of in-

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ABBREVIATIONS: REM, rapid eye movement; BLA, basolateral nucleus of the amygdala; CS, conditioned stimulus; LA, lateral nucleus of the amygdala; PI-3, phosphatidylinositol 3; LTP, long-term potentiation; REMD, rapid eye movement sleep deprivation; US, unconditioned stimulus; ITI, intertrial interval; MAPK, mitogen-activated protein kinase; P-Akt, phospho-Akt; ANOVA, analysis of variance; FK-506, tacrolimus.

terest to investigate whether PTEN plays any role in synaptic plasticity and memory formation. Here, we report that REMD impairs the retention of fear memory and present the first evidence that PTEN is involved in the REMD disruption of fear memory.

Materials and Methods

Surgery. Male Sprague-Dawley rats weighing 150 to 220 g were used. They were housed in group cages of four rats each in the air-conditioned vivarium with free access to food and water. Throughout the study, a 12:12-h light/dark cycle was maintained with lights on at 8 AM. Behavioral tests were performed in the light phase. All procedures adhered to the Guidelines for Care and Use of Experimental Animals of the National Cheng-Kung University (Tainan, Taiwan).

Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). When fully anesthetized, they were mounted on a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA), and two cannulae made of 22-gauge stainless-steel tubing (C313G; Plastic Products, Roanoke, VA) were implanted bilaterally into the lateral (LA) and basolateral (BLA) subregions of amygdala. A 28-gauge dummy cannula was inserted into each cannula to prevent clogging. The coordinates were anteroposterior, −2.3 mm; mediolateral, ± 4.5 mm; dorsoventral, −7.0 mm according to Paxinos and Watson (1986). Three jewelry screws were implanted over the skull serving as anchors, and the whole assembly was affixed on the skull with dental cement. The rats were monitored and handled daily and were given 7 days to recover. Animals were randomly divided into three groups for injection of antisense, sense, and scrambled oligonucleotides, respectively. Rats were injected bilaterally with 1 nmol (1 mM × 1 μl, each side) antisense, sense, or scrambled oligonucleotides into the lateral or basolateral nuclei of the amygdala at 24 and 1 h before receiving fear conditioning. The antisense phosphorothioate-modified oligonucleotide was a 20-mer complementary to human PTEN mRNA (5′-CTGCTAGCCTCTGGATTTGA-3′, beginning at position 2097, GenBank accession no. AA017584). The sequences for sense and scrambled oligonucleotides were 5′-TCAAATCCAGAGGCTAG-CAG-3′ and 5′-CTTCTGGCATCCGGTTTAGA-3′, respectively. Oligonucleotides were administered bilaterally at a rate of 1 μl/min using 28-gauge infusion cannulae that extended 1.0 mm from the base of the guide. The infusion cannulas were attached to a 10-μl Hamilton syringe, and after the infusion, the cannulae were left in place for 1 to 3 min to allow diffusion of the drugs from the tip.

Fear Conditioning. The startle response was measured in a startle apparatus (San Diego Instrument, San Diego, CA) as described previously (Lin et al., 2003a). In brief, rats were constrained in a Plexiglas cylindrical tube (length, 20 cm; diameter, 10 cm) with an accelerometer sensor attached on the base. The whole set-up was enclosed in a ventilated, sound-attenuating cabinet (length, 38 cm; width, 38 cm; height, 55 cm). The acoustic startle stimulus was 50-ms white noise at the intensity of 95 dB delivered by a speaker 30 cm above the animal. The visual conditioned stimulus (CS) was a 3.7-s light produced by an 8-W fluorescent bulb. The unconditioned stimulus (US) was a 0.6-mA foot shock with duration of 0.5 s. All data were reserved for offline analyses.

Acclimation. On 3 consecutive days, rats were placed in the startle test boxes for 10 min and then were returned to their home cages.

Matching. On 2 consecutive days, rats were placed in the startle box, and 3 min later, they were presented with 10 startle stimuli at 2-min intertrial intervals (ITIs). On the basis of their mean startle amplitudes in the second of these two sessions, rats were matched into groups with similar response levels.

Conditioning Training. Rats were placed in the startle boxes, and after a 5-min acclimation period, they received 10 light and foot shock pairings with an ITI of 2 min. Unpaired controls received the same number of light and foot shock presentation but in a pseudorandom fashion in which the US could occur at anytime except at 3.2 s after the CS.

Test. Twenty-four hours after training, rats were tested for fear-potentiated startle. This involved 30 startle-eliciting noise bursts presented alone (noise-alone trial) and 30 noise bursts presented 3.2 s after the onset of the 3.7-s light (light-noise trials). The two trial types were presented in a balanced mixed order (ITI, 30 s). The percentage of fear-potentiated startle was computed as follows: [(startle amplitude on CS-noise − noise-alone trials)/(noise-alone trials)] × 100.

REM Sleep Deprivation. Selective deprivation of REM sleep was accomplished by placing the rats on a small Plexiglas pedestal (8.5 cm in diameter) approximately 4 cm above the floor of the cage in which the animals were maintained (Smith et al., 1998). The floor was covered with water 3 cm deep. Food and water were available ad libitum. In this situation, the rats were unable to completely relax the large muscle groups without falling from the platform, getting wet, and waking. Rats in the REMD 1–12 group were dried with a towel, if necessary, and were returned to their home cages until testing. Rats in REMD 13–24 and 1–24 groups were dried and returned to their home cage to take a rest and relax for approximately half an hour and then were taken to the startle box for testing.

Western Blot Analysis. One hour after the test, rats were killed by decapitation. LA and BLA were sonicated briefly in ice-cold buffer (50 mM Tris-HCl, pH 7.5, 0.3 M sucrose, 5 mM EDTA, 2 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, and 4 μg/ml aprotinin). After sonication, the samples were centrifuged at 7500 rpm for 15 min, and the supernatant was obtained after pelleting the crude membrane fraction by centrifugation at 50,000 rpm for 1 h at 4°C. Protein concentration in the soluble fraction was then measured using a Bradford assay with bovine serum albumin as the standard. Equivalent amounts of protein for each sample were resolved in 8.5% SDS-polyacrylamide gels, blotted electrophoretically to Immobilon (Millipore, Bedford, MA), and blocked overnight in Tris-buffered saline (50 mM Tris-HCl, pH 7.5, and 150 mM NaCl) containing 3% bovine serum albumin. For detection of the protein level of PTEN and phosphorylated forms of Akt and MAPK, blots were incubated with anti-PTEN (1:2500), anti-phospho-Akt (1:2500) and anti-phospho-ERK (1:2500; all from Santa Cruz Biotechnology, Inc., Santa Cruz, CA) antibodies. An enhanced chemiluminescence kit (PerkinElmer Life and Analytical Sciences, Boston, MA) was used for detection. Western blots were developed in the linear range used for densitometry. The density of the immunoblots was determined by an image analysis system installed with the software BIO-ID (Vilber Lourmat, Marne-La-Vallée, France).

Results

Effect of REM Sleep Deprivation on the Retention of Fear Memory. Rats were given 10 pairings of light (CS) and foot shock (US). Immediately thereafter, they were divided into either REMD control or one of three REMD experimental groups. REM sleep was prevented in a 12-h period either immediately after the training session (REMD 1–12, group A) or 12 h later (REMD 13–24, group B). The remaining REMD group was deprived of REM sleep for a 24-h period (REMD 1–24, group A+B). Conditioned rats that were not given sleep deprivation served as REMD controls (Fig. 1A). Analysis of test scores after REMD is shown in Fig. 1B, which disclosed a significant effect of group ($n = 10$ rats for REM control, $n = 7$ rats for REM 1–12, $n = 7$ rats for REM 13–24, $n = 7$ rats for REM 1–24; $F_{3,27} = 5.64$, $P < 0.01$). The post hoc Newman-Keuls tests revealed that rats in the REMD 13–24

and REMD 1–24 groups displayed a significantly lower startle reflex compared with the control group ($P < 0.01$). In contrast, the startle amplitude of REMD 1–12 group was not different from that of control ($P > 0.5$). These results indicate that REMD impaired fear memory when administered during a 12-h period beginning at 13 h after training. Furthermore, to ensure that the levels of startle in response to the cue were specific for an association between the cue and the foot shock, a group of unpaired rats was subjected to REMD 13–24 (unpaired B, $n = 5$ rats). Figure 1B shows that there was no difference in startle amplitude between before and after REMD ($P > 0.1$) in unpaired rats, and startle response was not decreased to below the baseline level in these unpaired rats. The lack of the effect of REMD in unpaired rats suggests a selective effect of REMD on the learned association between CS and US but not on the startle reflex itself.

Effect of REMD on the Conditioning-Induced Protein Phosphorylation. We have shown previously that conditioned rats exhibited a transient increase in Akt phosphorylation after behavioral tests (Lin et al., 2001, 2003b). The increase was transient and significant from 60 to 120 min after test but not at other time points. Therefore, we examined whether the activated state of PI-3 kinase evoked by fear conditioning was affected after REMD. Rats were trained with fear-potentiated startle paradigm and subsequently were assigned to REMD control and three REMD experimental groups. Immunoblotting for phospho-Akt (P-Akt) was performed 60 min after test. Figure 1C shows a significant effect of group ($n = 5$ rats for each group, $F_{4,35} = 15.81$, $P < 0.0001$), and the degree of Akt phosphorylation was reduced in the REMD 13–24 and REMD 1–24 groups ($P < 0.001$). In contrast, Akt phosphorylation in REMD 1–12 group was comparable with and not significantly different from control group ($P = 0.25$). The change in phosphorylation was not a result of an alteration in total amount of Akt because expression of total Akt did not differ among the groups.

A similar pattern of results was obtained when measuring MAPK phosphorylation. Analysis of p42 MAPK phosphorylation revealed a significant effect of group ($n = 8$ rats for unpaired and REMD control and $n = 9$ rats for each REMD experimental group; $F_{4,38} = 6.50$, $P < 0.005$). As shown in Fig. 1D, p42 phosphorylation induced by fear training was significantly reduced in the REMD 13–24 and REMD 1–24 groups ($P < 0.01$) but not for REMD 1–12 group ($P = 0.15$). In addition, group comparison performed on p44 phosphorylation displayed significant difference among REMD groups ($F_{4,38} = 5.53$, $P < 0.005$). Fear testing-induced phosphorylation of p44 was significantly decreased in the REMD 13–24 and REMD 1–24 groups ($P < 0.01$) but not in the REMD 1–12 group. Taken together, these results suggest that memory test-induced protein phosphorylation was abrogated after REMD.

Effect of REMD on the Acquisition of Fear Memory. To investigate whether REMD affected the acquisition of fear memory, REMD was applied 24 h before training (Fig. 2A). Figure 2B depicts that all four groups displayed comparable amplitude of startle potentiation after training. The ANOVA for startle scores did not detect a significant effect for group ($n = 7$ rats for each group; $F_{3,24} = 0.02$; $P > 0.5$).

Western blotting analysis showed that pairing of light and foot shock induced phosphorylation of Akt and MAPK. However, the phosphorylated states of Akt ($n = 10$ rats for each REMD group; $F_{3,36} = 0.04$, $P > 0.5$) and MAPK ($n = 10$ rats for each REMD group; p42: $F_{3,36} = 0.15$, $P > 0.5$; p44, $F_{3,36} = 0.94$, $P > 0.5$) in all three REMD experimental groups were

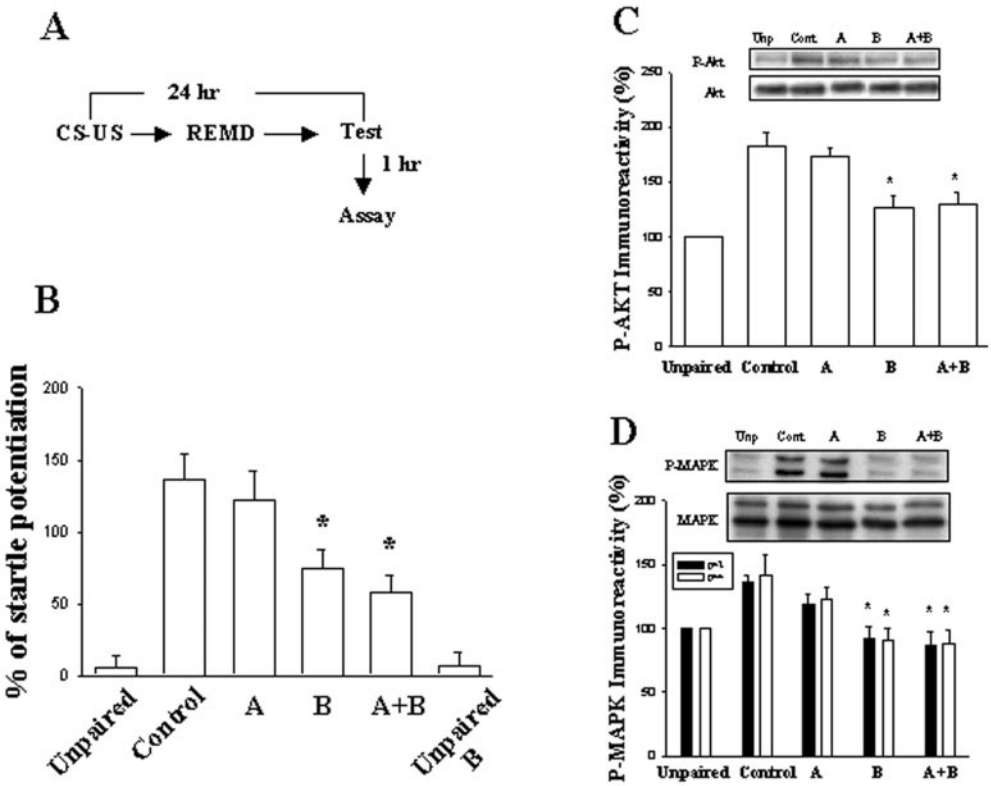

Fig. 1. REM sleep deprivation reduces fear-potentiated startle and memory retrieval-induced protein phosphorylation. A, behavioral procedure used in the following experiments. B, comparison of startle potentiation among control and three REMD groups. Startle potentiation was significantly reduced in the REMD 13–24 and REMD 1–24 groups. ⋆, $P < 0.01$ versus control. Note that there was no difference between control and REMD 13–24 in unpaired rats ($P > 0.1$), suggesting a selective effect of REMD on the learned association between CS and US but not on the startle reflex itself. C and D, representative blots and mean ± S.E. percentage of P-Akt (C) and P-MAPK (D) in control and three REMD groups. ⋆, $P < 0.01$ versus control.

not different from those of the control group (Fig. 2, C and D). Taken together, these results suggest that REMD before training has no effect on the acquisition of fear memory, as measured with fear-potentiated startle and protein phosphorylation.

Effect of REMD on the Akt and MAPK Phosphorylation in Unpaired Rats. To determine whether REMD affected the basal phosphorylated state of Akt and MAPK, rats were given light and foot shock in an unpaired manner. Immediately after training, rats were divided into either REMD control or one of three REMD experimental groups. P-Akt and P-MAPK were analyzed at 1 h after testing. We found that Akt phosphorylation in three experimental groups was comparable with and not significantly different from that of control group ($F_{3,16} = 1.10$, $P = 0.378$). Similar result was obtained with MAPK phosphorylation. There is no significant difference between experimental and control groups (p42: $F_{3,16} = 1.77$, $P = 0.193$; p44: $F_{3,16} = 1.57$, $P = 0.233$). Thus, REMD per se did not affect the phosphorylated state of Akt and MAPK in unpaired animals.

Mechanisms Underlying Impairment of Fear Memory by REMD. We have shown previously that giving light-alone trials to the animals without pairing with aversive foot shock caused extinction of memory, dephosphorylation of Akt, and an increase in calcineurin activity (Lin et al., 2003a,b). Therefore, calcineurin is a likely candidate responsible for the decrease in the phosphorylated state of Akt. We first assayed calcineurin activity by measuring the released inorganic phosphate from the phosphopeptide substrate that was insensitive to okadaic acid but could be blocked by FK-506. Rats received 10 pairings of light and foot shock and subsequently were assigned into REMD control and three REMD experimental groups. After testing, the release of inorganic phosphate from LA and BLA was measured in each group. We failed to detect any difference between control and any of three experimental groups ($n = 7$ rats for each group, $F_{3,24} = 0.01$, $P > 0.5$).

We tested whether calcineurin inhibitor affected REMD by giving FK-506 (10 μg dissolved in 2.0 μl dimethyl sulfoxide, 1.0 μl per side) bilaterally into the amygdala before REMD. This dose of FK-506 has been shown to block extinction of memory as well as dephosphorylation of Akt (Lin et al., 2003b). However, in the present study, microinjection of FK-506 into the amygdala did not affect REMD-induced depression of startle reflex in the REMD 13–24 and 1–24 groups. Analysis of startle scores revealed a significant effect of group ($F_{3,24} = 2.48$; $P < 0.05$). The post hoc Newman-Keuls tests showed that rats in the REMD 13–24 and REMD 1–24 groups displayed significantly less startle reflex compared with those in control and REMD 1–12 groups ($P < 0.05$). Thus, calcineurin is not involved in REMD-induced reduction of fear memory.

PTEN is a dual protein/lipid phosphatase that dephosphorylates the D3 position of PI-3 kinase product phosphatidylinositol 3,4,5-triphosphate and consequently down-regulates the effect of PI-3 kinase pathway (Maehama and Dixon, 1998). Therefore, it is reasonable to speculate that disruption of memory after REMD may be linked to PTEN. Rats were trained with fear-potentiated startle paradigm and subsequently subjected to REMD. After testing, the time course for expression of PTEN from the LA and BLA was quantified in each group. Figure 3A shows that immediately after testing, PTEN level in the REMD 13–24 group was significantly increased, which sustained for at least 2 h after test and subsided within 4 h ($F_{5,36} = 4.02$, $P < 0.01$). Newman-Keuls t tests disclosed that differences existed between control and <10 min, 1, and 2 h time points ($P < 0.05$). No significant difference was detected between control and 4 h or control and 8 h time points ($P > 0.05$). In contrast, in REMD control and 1–12 groups, PTEN immunoreactivities were not altered

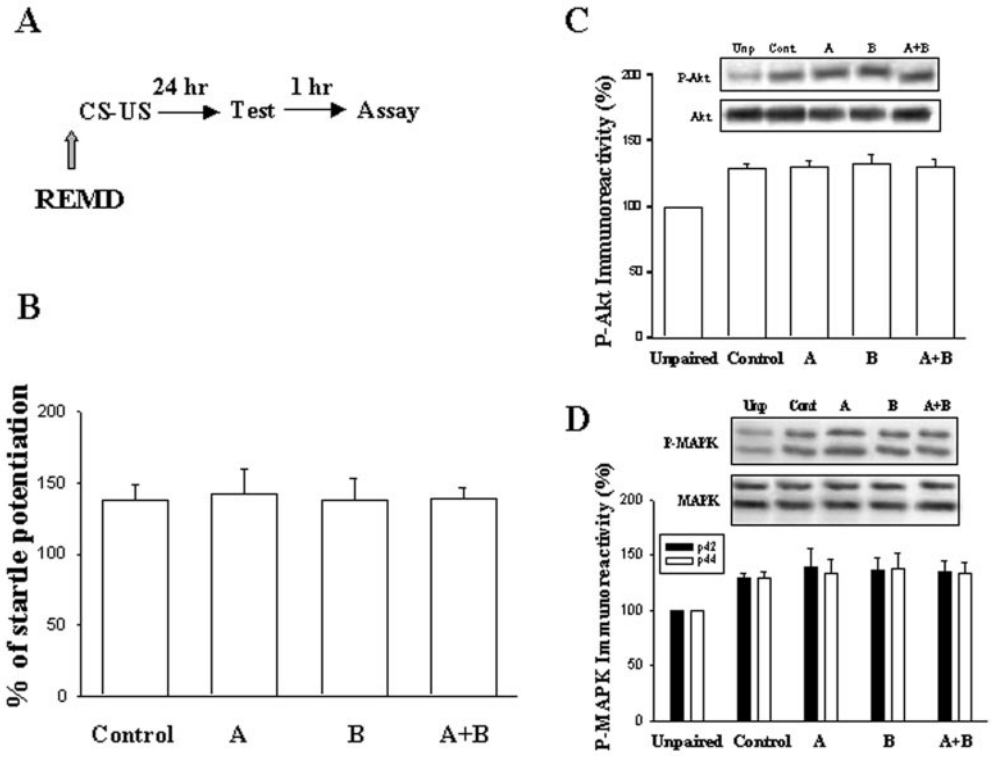

Fig. 2. Lack of the effect of REMD on the acquisition of fear memory. A, behavioral procedure used in the following experiments. B, comparison of startle potentiation among control and three REMD groups. REMD was applied before training. The degree of startle potentiation was not different between any two of four groups. C and D, representative blots and mean ± S.E. percentage of P-Akt (C) and P-MAPK (D) in control and three REMD groups. Fear training increased phosphorylation of Akt and MAPK compared with unpaired rats. However, the phosphorylated state of Akt and MAPK was not affected by REMD administered before training.

after test (Fig. 3, B and C). In addition, we repeated the experiments in the unpaired animals. After receiving light and shock in an unpaired manner, rats were subsequently given REMD 13–24. The result showed that PTEN levels in unpaired rats were not significantly different with or without REMD ($n = 5$ rats, $P > 0.05$) (Fig. 3D). These results clearly demonstrate that memory retrieval after REMD is accompanied with an increase in PTEN expression. To see whether PTEN expression was associated with REMD, per se, PTEN levels were measured at various time points after REMD 13–24 but without testing. Figure 4 shows that PTEN levels were increased after REMD, which persisted for at least 2 h ($n = 5$ rats).

To test whether down-regulation of PTEN in the amygdala would reverse REMD-induced memory deficit, we used antisense oligonucleotide to knock down PTEN expression. Bilaterally cannulated rats were infused with PTEN antisense, sense, or scrambled oligonucleotides at 1 and 24 h before training. PTEN antisense oligonucleotide was designed according to previous study (Butler et al., 2002), in which elimination of PTEN by >90% has been reported in α-mouse liver-12 cells. Systemic administration of PTEN antisense oligonucleotide once a week in mice suppressed PTEN mRNA and protein expression in liver and fat by up to 90 and 75%, respectively. As illustrated in Fig. 5, PTEN expression was different among REMD groups in sense ($F_{3,20} = 12.03$, $P < 0.001$, Fig. 5A) or scrambled ($F_{3,24} = 6.59$, $P < 0.005$, Fig. 5B) oligonucleotide-treated animals. REMD-induced PTEN expression was significantly increased in REMD 13–24 and REMD 1–24 groups relative to REMD control ($P < 0.01$) and REMD 1–12 groups ($P < 0.01$). In contrast, pretreatment with antisense oligonucleotide abolished REMD-induced increase in PTEN (Fig. 5C). ANOVA analysis did not show any difference among control and REMD groups ($F_{3,20} = 2.07$, $P = 0.14$). It is noted that PTEN levels were not decreased to below the control level (PTEN level in conditioned rats that were not subjected to sleep deprivation) in antisense oligonucleotide-treated rats after REMD. This result suggests that at the dose used, antisense oligonucleotide had a selective effect on sleep deprivation-induced PTEN expression but not on the constitutive expression of PTEN. It is interesting for the future study to examine whether higher doses of antisense oligonucleotide would reduce baseline level of PTEN and facilitate fear-potentiated startle.

Further support for an involvement of PTEN came from the observation that decrease in the phosphorylated state of Akt and MAPK after REMD could be prevented by antisense oligonucleotide treatment, whereas sense and scrambled oligonucleotides were without effect (Fig. 6).

Quantification of startle potentiation 24 h after training revealed a block of REMD-induced depression of startle reflex in REMD 13–24 and REMD 1–24 groups by antisense oligonucleotide (Fig. 7A). ANOVA analysis did not detect any difference between control and three REMD groups ($F_{3,20} = 0.16$, $P = 0.92$). On the other hand, those receiving sense ($F_{3,24} = 6.60$, $P < 0.005$) or scrambled ($F_{3,20} = 13.8$, $P < 0.001$) oligonucleotides displayed a normal depression of startle. Thus, antisense oligonucleotide for PTEN blocked REMD-induced startle reduction at the same dose that inhibited Akt and MAPK dephosphorylation. The infusion cannula tip locations are shown in Fig. 7B. Only rats with cannula tips at or within the boundaries of LA and BLA were included in the data analysis.

Finally, it is possible that the observed deficits in learning tasks after REMD may merely reflect deficits in performance at retest because of fatigue or depressed motivation. To rule out this possibility, additional experiments were performed in which memory retention was tested 1 day after REMD (2 days after training). A one-way ANOVA showed that group effect was statistically significant ($F_{3,20} = 3.72$, $P < 0.05$).

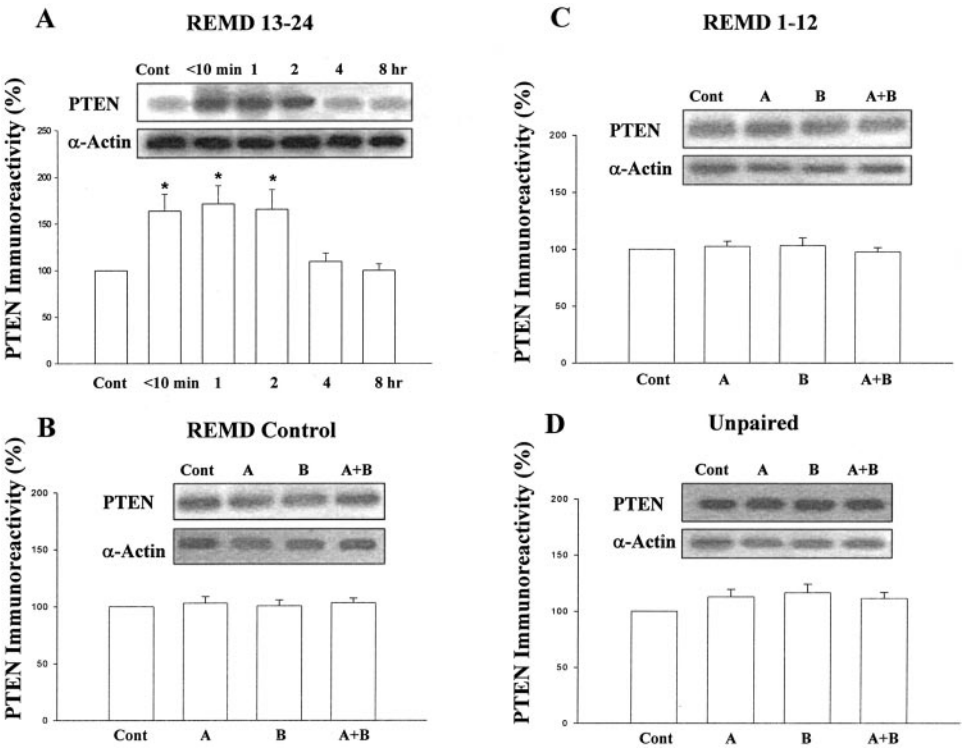

Fig. 3. REMD induces an increase in the expression of PTEN. A, time course of PTEN expression induced by REMD 13–24. The representative blots and mean ± S.E. of PTEN immunoreactivities from rats decapitated at various time points ($n = 6$ rats in each time point) after REMD 13–24 are shown (α-actin used as internal control). ⋆, $P < 0.05$ versus control. B and C, PTEN expression is not changed in control (B) and REMD 1–12 (C) groups. D, PTEN levels in unpaired rats which were subjected with REMD 13–24 were not significantly different from those without REMD.

Post hoc tests showed that the rats in REMD 13–24 and REMD 1–24 groups exhibited less startle reflex than those of REMD control and REMD 1–12 groups ($P < 0.02$) (Fig. 8).

Discussion

It has been hypothesized for many years that sleep is important for neuronal plasticity and memory (Fishbein and Gutwein, 1977; Pearlman, 1979; Smith, 1996); memory storage for different behavioral tasks is impaired when REM sleep deprivation follows training. However, the molecular processes underlying sleep deprivation-induced memory impairment remain unknown. In the present study, we found that REMD in a 12-h period beginning at 13 h after training reduced the retention of fear memory. Fear conditioning-induced phosphorylation of Akt and MAPK in the rat amygdala was abrogated after REMD. More importantly, REMD-induced impairment of fear memory and dephosphorylation of P-Akt and P-MAPK was inhibited by PTEN antisense but not by sense or scrambled oligonucleotides and was concurrently accompanied by a time-dependent increase in the expression of PTEN in the amygdala. These results provide the first evidence suggesting that under normal conditions, REM sleep is crucial for normal acquisition of learning and memory, and post-training REM sleep deprivation impairs memory by activation of PTEN, which reduces the phosphorylation of Akt, a process required for the consolidation of memory.

REMD Impairs Retention of Fear Memory. It has been suggested that reprocessing of newly acquired material within the neural networks during sleep significantly contributes to the formation of different types of memory (Wilson and McNaughton, 1994; Hirase, 2001; Hoffman and McNaughton, 2002). Recent studies have shown that the amygdale and hippocampus may be platform for sleep-dependent memory processing (Graves et al., 2001; Louie and Wilson, 2001; Ribeiro et al., 2002). Here, we support this assumption

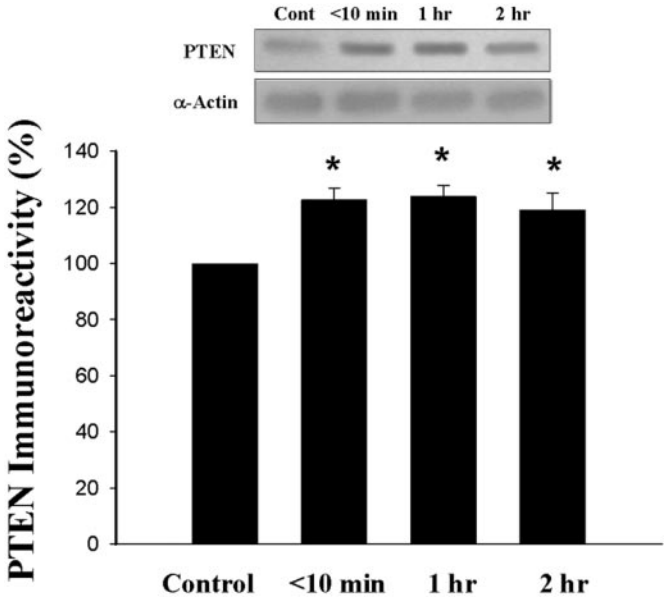

Fig. 4. Increase in the expression of PTEN after fear training. Rats were exposed to a light repeatedly paired with an aversive foot shock. Protein extracts from the LA and BLA were analyzed with Western blotting at different time points after training.

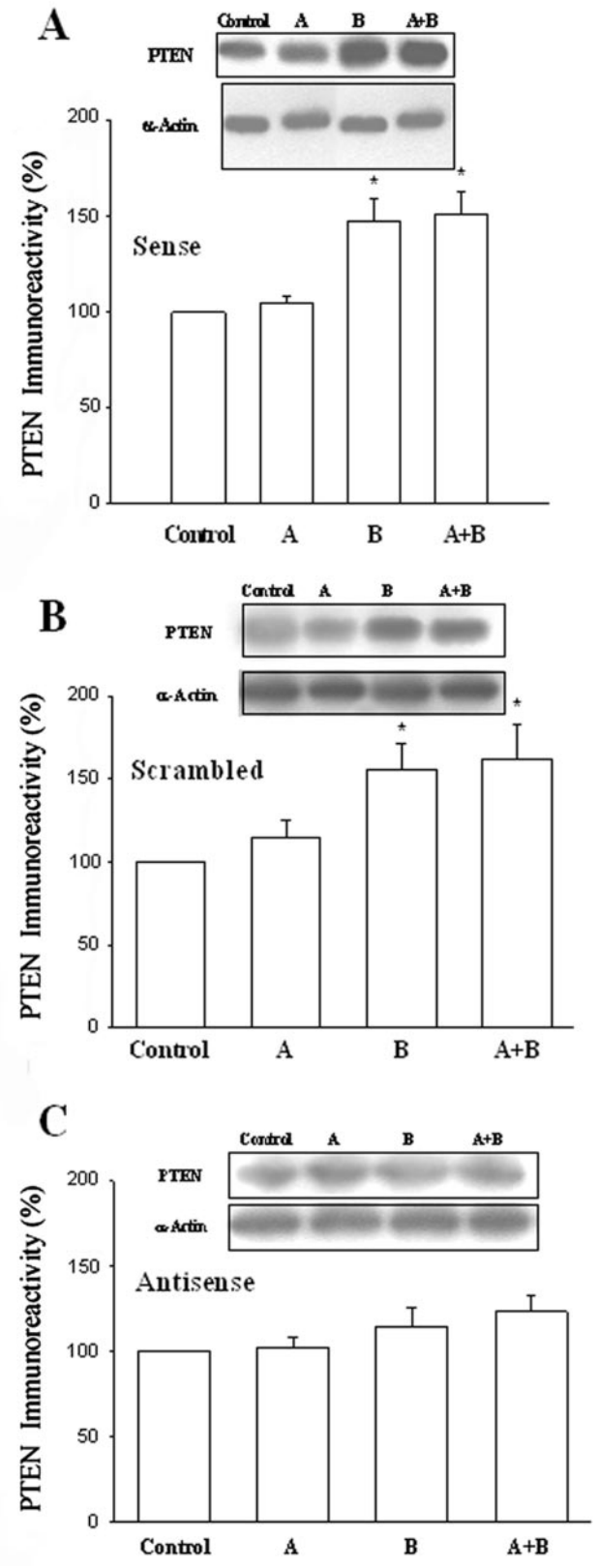

Fig. 5. Block of PTEN expression by antisense but not by sense or scrambled oligonucleotides. In sense (A) or scrambled (B) oligonucleotide-treated animals, REMD induced a normal PTEN expression in REMD 13–24 and REMD 1–24 groups. C, in contrast, pretreatment with antisense oligonucleotide abolished REMD-induced increase in PTEN.

by showing that REMD at specific intervals after training impairs memory retention measured with fear-potentiated startle. The vulnerable time period for REMD-induced memory deficit was at 13 to 24 h after training. The time window of 13 to 24 h after training was unique in that, in other forms of memory tasks such as Morris water maze and radial arm maze, REM window was usually at somewhere between 1 to 12 h after training (Smith and Rose, 1996; Smith et al., 1998). However, previous studies have shown that the more trials given in a single session, the shorter the latency to onset of the REM window (Smith, 1996). Thus, it would be of interest to examine whether the latency to onset of REM window decreased as the number of training trials per session increased. On the other hand, the latency to onset of paradoxical sleep window could vary considerably depending on the type of learning task.

It was argued that the effects of REMD on memory could be accounted for by stress or other factors. In the present study, the memory deficit was observed in one interval (REMD 13–24) but not the other (REMD 1–12), suggesting that stress may not be the primary factor influencing memory. If stress was the main factor contributing to REMD-induced memory impairment, one might expect that a longer period of stress would induce a greater memory deficit. However, the deficits observed in Fig. 1 which involved 12-h and 24-h periods of REMD did not reach statistical significance ($t_{12} = 0.97$, $P = 0.35$). Thus, in the present study, stress seems not to be the primary factor responsible for memory deficient effect of REMD. Consistent with this notion, La Hoste et al. (2002) found learning deficit after REMD when they controlled the release of stress hormone by using adrenalectomy combined with corticosterone pellet implantation. Furthermore, the effect was unlikely to have been caused by performance deficits at retest because the effect was noted 1 day after REMD, which would seem to provide ample time for the secondary effects (fatigue and motivation) to subside.

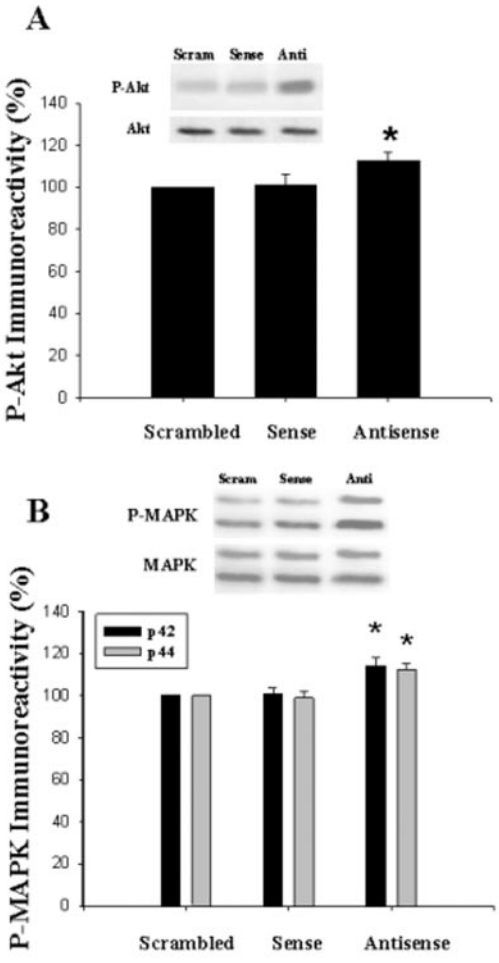

Fig. 6. Block of REMD-induced reduction of protein phosphorylation by PTEN antisense oligonucleotide. Rats were given PTEN antisense, sense, or scrambled oligonucleotides bilaterally into the amygdala at 1 and 24 h before training. Phosphorylated Akt (A) and MAPK (B) were measured at 1 h after testing. REMD-induced reduction of protein phosphorylation normally seen in REMD 13–24 was absent in the antisense-treated animals.

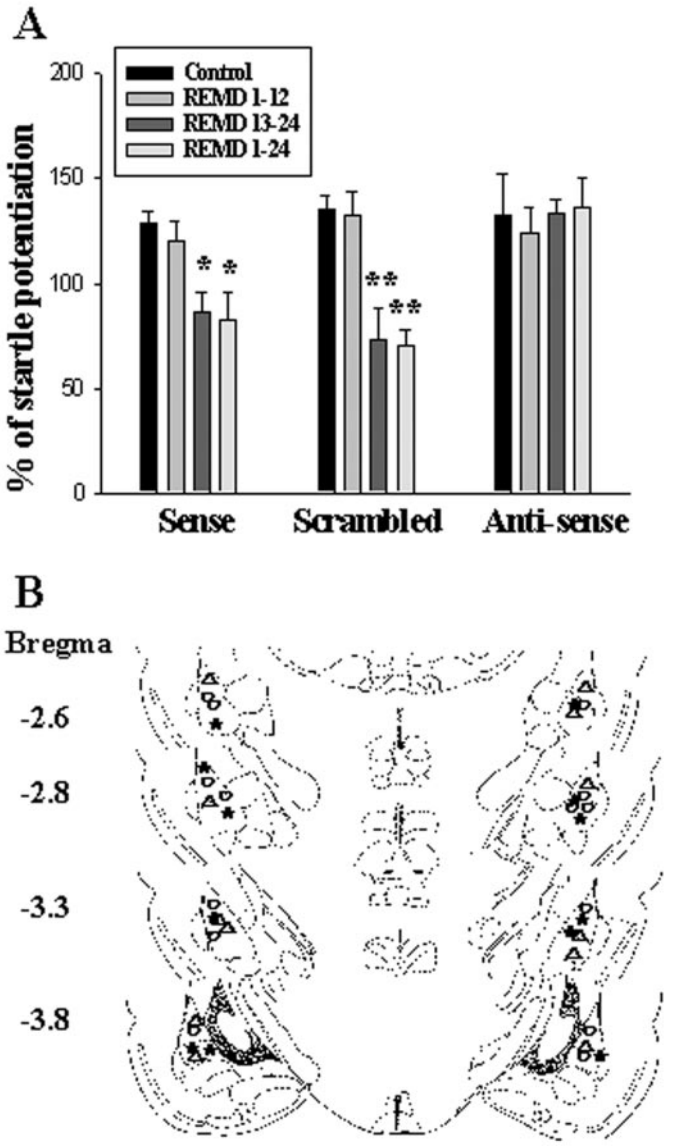

Fig. 7. REMD-induced depression of fear-potentiated startle is blocked by PTEN antisense oligonucleotide. A, rats were given PTEN antisense, sense, or scrambled oligonucleotides bilaterally into the amygdala at 1 and 24 h before training. Fear-potentiated startle was measured 24 h after training. REMD-induced depression of startle reflex normally seen in REMD 13–24 and REMD 1–24 groups was absent in the antisense-treated animals. ⋆, $P < 0.005$; ⋆⋆, $P < 0.001$ versus control. B, cannula tip placements from rats infused with sense (○), scrambled (△), or antisense (⋆) oligonucleotides.

Involvement of PTEN in the Disruptive Effect of REMD on Fear Memory. When learned association was retrieved from long-term memory storage by behavioral tests, a process of reconsolidation was initiated, which reactivated signal cascades involved in the initial consolidation of memory (Hall et al., 2001; Lin et al., 2003a,b). In agreement with these results, memory retrieval in fear-conditioned rats resulted in the synchronization of amygdala theta rhythm (Seidenbecher et al., 2003). Rats in the paired but not unpaired groups displayed fear-potentiated startle and a selective increase in the phosphorylation of Akt and MAPK in the amygdala after retrieval of fear memory. Paired and unpaired groups received the same number of CS and US presentations during training procedure but differed in the correlation of pairing, suggesting that retrieval-induced activation of Akt and MAPK is specific to the learning component of task. These results indicate that Akt and MAPK, known to be required for fear memory acquisition, are also activated by phosphorylation during memory retrieval.

A fully consolidated memory, when retrieved or reactivated, could become fragile and sensitive to disruption by amnestic agents (Nader et al., 2000). The recall of memory thus seems to place it into a labile state, from which it is reconsolidated back into a stable form. It is well established that the amygdale plays a critical role in fear-memory formation. Using positron emission tomography, it has been shown that some brain areas were more activated than the rest of the brain during REM sleep (Maquet et al., 1996). In particular, limbic forebrain structures and the amygdale were activated, which might lead to reactivation of affective components of memory. Therefore, the impairment of fear memory after REMD could be the results of loss of the enhancement of retrievability and reconsolidation during REM. In addition, the expression of PTEN counteracted the reactivation of PI-3 kinase and its downstream signal cascades, resulting in the block of reconsolidation of fear memory.

How can a biochemical change in response to behavioral manipulations be large if, presumably, it is restricted to a small subset of synapses that hold the membrane trace? It is likely that the subsets of cells involved may be larger than expected. Recent anatomical data showed that larger numbers of LA and BLA neurons were affected after fear conditioning (Stanciu et al., 2001; Rattiner et al., 2004). Furthermore, it is believed that LA and BLA have reciprocal connections providing the amplification mechanism during fear conditioning. Indeed, previous studies have successfully measured the changes in protein phosphorylation and *N*-methyl-D-aspartate subunit protein after fear conditioning (Schafe et al., 2000; Lin et al., 2001; Zenebi et al., 2003; Rattiner et al., 2004). In summary, a long history of human and animal research has shown that REMD after learning results in memory deficits, although the underlying mechanism is not known. Our data have shown two important aspects: fear retrieval-induced phosphorylation of Akt and MAPK was abrogated after REMD, and REMD-induced memory impairment was associated with a time-dependent increase in the expression of PTEN in the amygdala. Given the known role of PI-3 kinase activation in synaptic plasticity (Lin et al., 2001; Sanna et al., 2002; Man et al., 2003), these findings suggest that down-regulation of PI-3 kinase by PTEN underlies REMD-induced memory impairment.

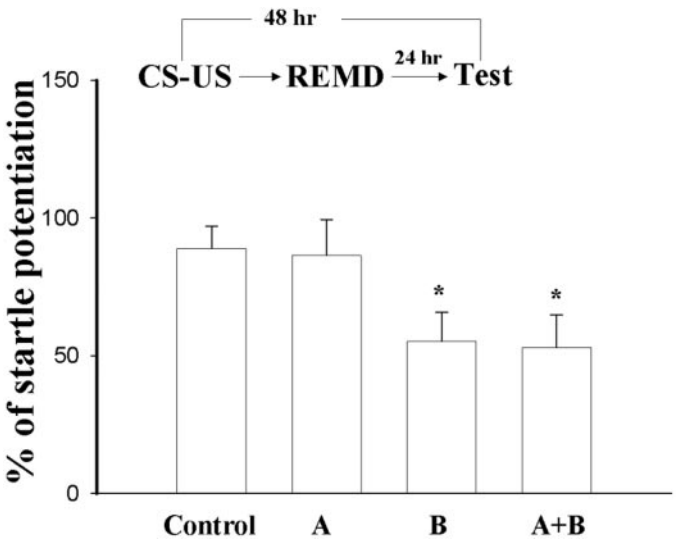

Fig. 8. REM sleep deprivation still reduces fear-potentiated startle when tested 24 h after sleep deprivation. Comparison of startle potentiation among control and three REMD groups. Startle potentiation was significantly reduced in the REMD 13–24 and REMD 1–24 groups. ⋆, $P < 0.02$ versus control.

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