Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 8699–8704, August 1996
Neurobiology

Mice lacking the gene encoding tissue-type plasminogen activator show a selective interference with late-phase long-term potentiation in both Schaffer collateral and mossy fiber pathways

YAN-YOU HUANG*, MARY ELIZABETH BACH*, HANS-PETER LIPP†, MIN ZHUO*, DAVID P. WOLFER†, ROBERT D. HAWKINS*, LUC SCHOONJANS‡, ERIC R. KANDEL*, JEAN-MARIE GODFRAIND§, RICHARD MULLIGAN¶, D. COLLEN‡, AND PETER CARMELIET‡

*Howard Hughes Medical Institute, Center for Neurobiology and Behavior, College of Physicians and Surgeons of Columbia University and New York State Psychiatric Institute, 722 West 168th Street, New York, NY 10032; †Anatomisches Institut, Universität Zürich-Irchel, Zürich, Switzerland; ‡Center for Transgene Technology and Gene Therapy, Vlaams Interuniversitair Instituut voor Biotechnologie, Katholicke Universiteit Leuven, Campus Gasthuisberg O and N, Herestraat 49, B-3000 Louvain, Belgium; §Department of Physiology and Pharmacology, Système Nerveux, Université de Louvain, Louvan-la-Neuve, Belgium; and ¶Whitehead Institute, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA 02142

Contributed by Eric R. Kandel, April 12, 1996

ABSTRACT **The gene encoding tissue-type plasminogen activator (t-PA) is an immediate response gene, downstream from CREB-1 and other constitutively expressed transcription factors, which is induced in the hippocampus during the late phase of long-term potentiation (L-LTP). Mice in which the *t-PA* gene has been ablated (t-PA$^{-/-}$) showed no gross anatomical, electrophysiological, sensory, or motor abnormalities but manifest a selective reduction in L-LTP in hippocampal slices in both the Schaffer collateral-CA1 and mossy fiber-CA3 pathways. t-PA$^{-/-}$ mice also exhibit reduced potentiation by cAMP analogs and D1/D5 agonists. By contrast, hippocampal-dependent learning and memory were not affected in these mice, whereas performance was impaired on two-way active avoidance, a striatum-dependent task. These results provide genetic evidence that t-PA is a downstream effector gene important for L-LTP and show that modest impairment of L-LTP in CA1 and CA3 does not result in hippocampus-dependent behavioral phenotypes.**

The hippocampus of the mammalian brain contains three major neural pathways that undergo an activity-dependent change in synaptic strength, called long-term potentiation (LTP). In each of these pathways, hippocampal LTP consists of two components, an early, transient, transcriptionally-independent component [early-phase LTP (E-LTP)] and a later, persistent, long-term component [late-phase LTP (L-LTP)] that requires protein and mRNA synthesis (1–3). Hippocampus-dependent long-term memory (4, 5), unlike short-term memory, also requires protein and mRNA synthesis, which implies that long-term memory requires the expression of specific genes and proteins (6, 7). The gene transcription required for long-term memory is thought to result from a learning-induced increase in neuronal activity, which ultimately leads to the strengthening of preexisting synapses as well as the formation of new synaptic connections. Evidence from *Aplysia*, *Drosophila*, and mice indicates that the activation of transcription involves recruitment of the constitutively active transcription regulator CREB-1. This raises the question: What are the genes that are downstream from this transcription regulator?

In the search for immediate response genes downstream from CREB-1, Qian *et al.* (8) identified tissue-type plasminogen activator (t-PA) as a protein encoded by an immediate-early gene, which is induced by an increase in neuronal activity. t-PA is an extracellular serine protease (9) that is induced in the hippocampus during LTP (8, 10, 11). Since the release of t-PA correlates with the morphological differentiation of neurons, the increased expression of t-PA may play a role in transcriptionally dependent synaptic plasticity and in the growth of new synaptic connections (10–12). To study the role of t-PA in long-term synaptic plasticity and hippocampal-dependent memory, we examined the effect of ablating the *t-PA* gene on LTP (13). We found that ablation of t-PA produces a partial reduction of L-LTP in both the Schaffer collateral and the mossy fiber pathways without altering the early phase of L-LTP. These experiments provide the first genetic identification of a downstream effector gene important for L-LTP. Partial disruption of L-LTP in t-PA$^{-/-}$ mice, however, produced no interference with hippocampus-dependent long-term memory but did produce a deficit in two-way active avoidance, a striatum-dependent task.

MATERIALS AND METHODS

Electrophysiology. Transverse hippocampal slices (400–500 μM) were placed in an interface chamber and perfused continuously with solution containing 124 mM NaCl, 1.3 mM $MgSO_4$, 4 mM KCl, 1.0 mM Na_2HPO_4, 2.0 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose. The perfusion solution was bubbled with 95% O_2 and 5% CO_2 at a flow rate of 1.5–2 ml/min. The temperature of the recording chamber was maintained at either 28°C or 32°C in different experiments. A stainless steel electrode was used for field excitatory postsynaptic potential (EPSP) recording, and a fine tungsten bipolar electrode was used for stimulation. The position of the electrodes for stimulating and recording in CA1 and CA3 was the same as described (2, 14). Test stimuli were of 0.05-ms pulse duration and 0.016-Hz frequency. LTP was induced by one or four trains of tetanus at the same intensity as test stimuli (100 Hz for 1 s, pulse duration 0.05 ms) in the Schaffer collateral-CA1 pathway and four trains of tetanus (100 Hz for 1 s) with double-pulse duration (0.1 ms) during tetanus in the mossy fiber-CA3 pathway. The initial slope of the EPSP was measured, and LTP was calculated as percentage change from the baseline EPSP slope. The following drugs were made and stored as concentrated stock solutions and were diluted 1000-fold when applied to the perfusion solution: 100 mM adenosine 3′,5′-cyclic monophosphorothioate (Sp-cAMPS; BioLog Life Sciences Institute, Bremen, Germany); 50 mM forskolin (Sigma; in dimethyl sulfoxide); 25 mM (±)APV (Research Biochemicals); and 50 mM *R*-(+)-6-bromo-APB (Research Biochemicals; in dimethyl sulfoxide).

Behavior. *Novel exploration.* The activity level of t-PA$^{-/-}$ (n = 6 for each gender) and wild-type (n = 6 for each gender) mice between the ages of 12 and 14 weeks was measured in a novel environment across 10 min using the Automex II system

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LTP, long-term potentiation; E-LTP, early-phase LTP; L-LTP, late-phase LTP; t-PA, tissue-type plasminogen activator; EPSP, excitatory postsynaptic potential; PTP, posttetanic potentiation; NMDA, *N*-methyl-D-aspartate; u-PA, urokinase-type plasminogen activator; CRE, cAMP-responsive element; Sp-cAMPS, adenosine 3′,5′-cyclic monophosphorothioate.

(Columbus Instruments, Columbus, OH). For each mouse, a clean Plexiglas cage was placed atop the Automex system, which measures capacitance changes produced by animal movements and yields a gross index of activity that does not distinguish between horizontal or vertical movements.

Conditioned fear. On the training day, t-PA$^{-/-}$ (n = 6 each gender) and wild-type (n = 6 each gender) mice were placed in a novel context, a mouse operant chamber (Med Associates). Three minutes after the mouse was placed in the chamber, a 20-s, 75-dB Sonalert tone was presented. During the last second of the tone, a 0.75-mA scrambled footshock was delivered through the grid floor. The footshock was followed by two more tone-shock pairings at 1-min intervals. Memory for the context-shock association was assessed 24 h later by measuring the amount of freezing exhibited by the mice when they were placed back in the same context, and memory for the tone-shock association was assessed in the presence of the tone in a second novel context that differed in terms of visual and olfactory cues. The mice were placed back into the old context for 3 min, and then 2 h later they were placed into the novel context for 3 min, followed by three 20-s tone presentations, each separated by a 1 min interval. Freezing, defined as a total lack of movement with the exception of respiration, was measured by an observer blind to the genotype of the mice.

Barnes circular maze. Male t-PA$^{-/-}$ (n = 6) and wild-type (n = 6) mice between 12 and 14 weeks of age were tested on the Barnes maze by an observer who did not know their genotype. The Barnes maze is a white disk with 40 holes cut in the perimeter. A long dark escape tunnel is placed underneath one of the holes. By entering the tunnel, the mouse escapes the open, brightly lit maze, which is associated with an aversive noise. The mice navigate to the escape tunnel using the relationships between distal cues in the room. The mice were tested once a day for 35 days until they met a criterion of seven out of eight consecutive days with three or fewer errors. Errors were defined as searching any hole that did not have the tunnel beneath it. The distance from the tunnel during the initial search was calculated by counting the number of holes between the first hole searched and the escape tunnel. On the first day of testing, the mouse was placed in the tunnel and left there for 1 min. One minute later, the first session commenced. At the beginning of each session, the mouse was placed in the middle of the maze in a 10-cm high cylindrical black start chamber, and a buzzer (80 dB) from a timer (Gradlab model 167) was turned on. The timer was affixed to the ceiling over the maze. After 10 s had elapsed, the start chamber was lifted and the mouse was free to explore the maze. The session ended when the mouse entered the escape tunnel or after 5 min elapsed. The tunnel was always located underneath the same hole, which was randomly determined for each mouse.

Morris Water Maze. t-PA$^{-/-}$ (12 females, 22 males) and wild-type mice (12 females, 22 males) between 10 and 14 weeks of age were tested in the Morris water maze by an observer who did not know their genotype. The mice underwent five consecutive daily training sessions of six trials per day. The intertrial interval varied from 30 to 45 min, and a trial lasted maximally 120 s. For further details see Schöpke *et al.* (15). The platform location was changed after day 3. If the mice employed a spatial strategy to find the platform, then on day 4 they would cross the previous location of the platform more frequently than the analogous locations in the other quadrants.

Active avoidance. t-PA$^{-/-}$ (10 females, 20 males) and wild-type mice (10 females, 20 males) between the ages of 10 and 14 weeks were tested in computer-operated two-way shuttleboxes by an observer who did not know their genotype. The mice underwent five consecutive training sessions of 80 trials per day with intertrial intervals that varied between 5 and 15 s. The duration of the conditioned stimulus (light) was 20 s, the maximum duration of the unconditioned stimulus (electric shock) was 10 s, and shock intensity was 150 μA. The animal could avoid the shock during the 10-s light period or escape it during the 10-s shock period by running to the other half of the box.

RESULTS

t-PA$^{-/-}$ Mice Have a Selective Defect in L-LTP in the Schaffer Collateral Pathway. We first examined two forms of short-term synaptic plasticity in the Schaffer collateral-CA1 pathway: posttetanic potentiation (PTP) and paired-pulse facilitation. In the presence of the *N*-methyl-D-aspartate (NMDA) antagonist, 2-amino-5-phosphonopentanoic acid (25 μM), a single train of 100 Hz for 1 s induced PTP that lasted 1–2 min and was comparable in amplitude and duration in both wild-type and t-PA$^{-/-}$ mice (Fig. 1*A*). There was also no difference between t-PA$^{-/-}$ and wild-type mice in paired-pulse facilitation (Fig. 1*B*) and in basal synaptic transmission and the threshold for the field EPSPs.

Earlier work in rats demonstrated that one high-frequency train of stimuli produces E-LTP that lasts longer than 1 h but less than 3 h and is independent of protein synthesis. By contrast, three to four high-frequency trains of tetanization produces L-LTP that lasts 8–10 h and is blocked by inhibitors of translation or transcription (1–3). We compared E-LTP and L-LTP in wild-type and t-PA$^{-/-}$ mice using two tetanization protocols: one

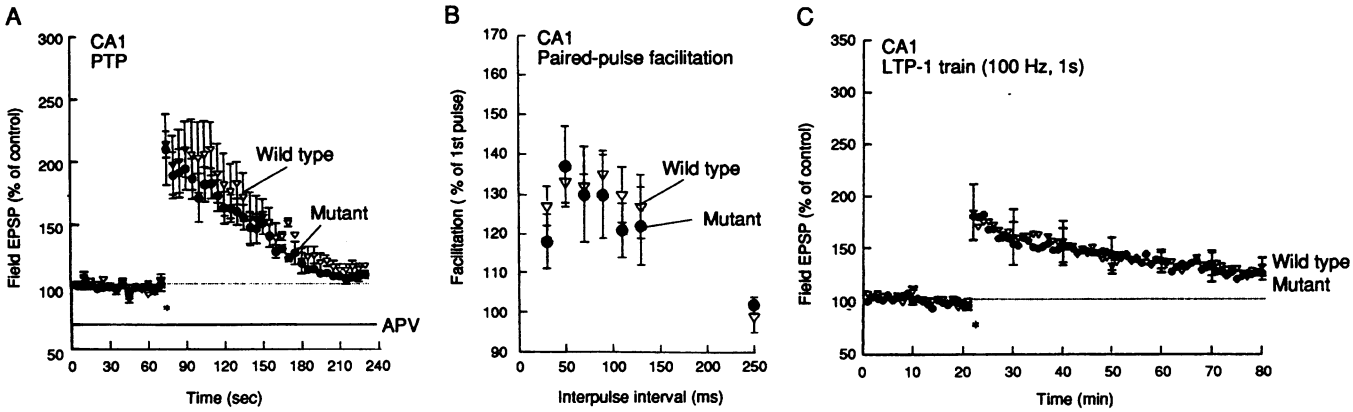

FIG. 1. Short-term synaptic plasticity and E-LTP in the CA1 region of t-PA wild-type and mutant (t-PA$^{-/-}$) mice. (*A*) PTP. A single train of tetanus (100 Hz for 1 s, 0.05-ms pulse duration) induces PTP in the presence of the NMDA antagonist 2-amino-5-phosphonopentanoic acid (APV; 25 μM). The field EPSP was sampled every second. The graph plots the percent change in the initial slope of the EPSP (mean ± SEM). No difference was observed in either the amplitude or time course of PTP between wild-type (open triangles) and mutant mice (closed circles) (n = 5 each). (*B*) Paired-pulse facilitation. There was no difference between t-PA wild-type and mutant mice comparing the EPSP produced by the second pulse and the first pulse in a pair as a function of the time interval between the pulses (n = 5 each). (*C*) E-LTP induced by a single train of tetanus. There was no difference in E-LTP between wild-type and mutant mice (n = 7 each). The same range of baseline EPSP slopes was used for the two groups (0.40–0.60 mV/ms).

train of 100 Hz for 1 s to produce only E-LTP and four trains to produce both E-LTP and L-LTP. In mutant mice, LTP induced by one train is normal and indistinguishable from LTP in wild-type mice (Fig. 1*C*). By contrast, four trains, which induced L-LTP in wild-type mice, induced potentiation that began to decay 1 h after tetanization in t-PA$^{-/-}$ mice. Thus, after 3 h, L-LTP was only 125 ± 19% in t-PA$^{-/-}$ mice, whereas it was 200 ± 15% in wild-type mice (n = 9 each, $p < 0.025$, Student's t test) (Fig. 2*A*). Low-frequency testing alone revealed no decay in the baseline EPSP in t-PA$^{-/-}$ mice (Fig. 2*B*). The defect in L-LTP in t-PA$^{-/-}$ mice also occurred in picrotoxin-treated slices (20 μm; n = 2; data not shown), suggesting it is independent of inhibition mediated by γ-aminobutyric acid.

Several lines of evidence suggest that this defect in L-LTP in t-PA$^{-/-}$ mice is specific. There is no comparable defect in L-LTP in mice deficient in urokinase-type plasminogen activator (u-PA$^{-/-}$; ref. 9) (Fig. 2*C*). The defect in L-LTP in t-PA$^{-/-}$ mice is probably not due to a defect in the NMDA receptor, because E-LTP was normal. In addition, analysis of the brains from wild-type, t-PA$^{-/-}$, and u-PA$^{-/-}$ mice for neuroanatomical abnormalities in fiber architecture (Liesegang's silver stain) (16), cortical lamination (Nissl stain), and hippocampal mossy fiber architecture (Timm's stain) revealed no qualitative differences between the different genotypes (four mice tested per genotype; data not shown). These results suggest that t-PA is critically involved in L-LTP. We have obtained independent support for this idea in experiments in which we found that exposing rat slices to recombinant t-PA protein facilitates L-LTP and that an inhibitor of t-PA can block L-LTP (17).

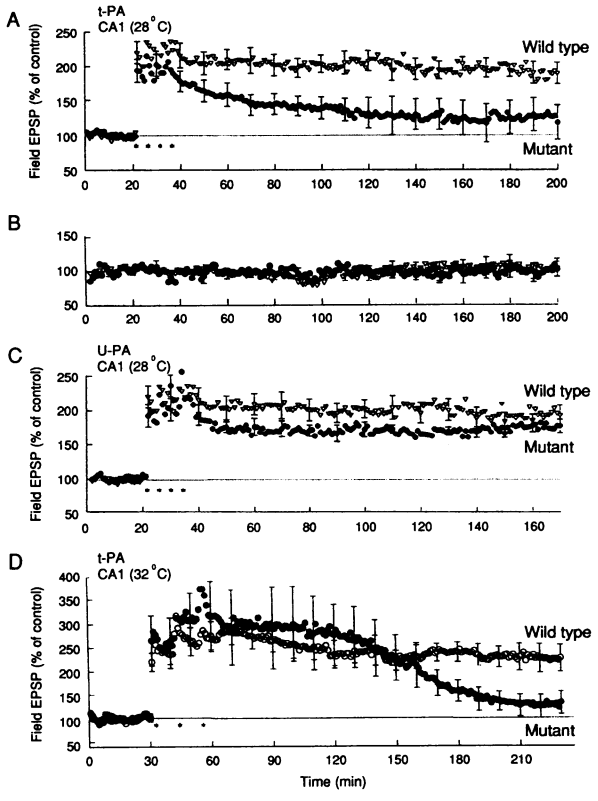

FIG. 2. L-LTP in the CA1 region of t-PA wild-type and mutant mice. (*A*) Four trains of tetanus (100 Hz for 1 s, 0.05-ms pulse duration, 5-min intervals between trains) induced a stable, long-lasting potentiation in wild-type mice (open triangles). The same tetanization induced a slowly decaying LTP in t-PA$^{-/-}$ mice (closed circles) that was significantly different from that in wild-type mice at 3 h (n = 9 each, $p < 0.01$). (*B*) Low-frequency stimuli alone did not induce any decay of the field EPSP slope in t-PA$^{-/-}$ mice (open triangles) compared with wild-type mice (closed circles) (n = 4 each). (*C*) There was no significant difference in L-LTP induced by four trains of tetanus between wild-type (open triangles) and u-PA$^{-/-}$ mice (closed circles) (n = 5 each). (*D*) L-LTP was examined in slices maintained at 32°C. LTP induced by three strong tetani (100 Hz for 1 s, 0.1-ms pulse duration, 10-min intervals) in t-PA$^{-/-}$ mice was smaller than that in wild-type mice 3 h after tetanus (n = 4 each, $p < 0.01$).

Because induction of LTP is affected by temperature and by the intensity of the tetanic stimulus, the defect in L-LTP in t-PA$^{-/-}$ mice was studied at a higher temperature (32°C rather than 28°C) with a stronger tetanus protocol (double-pulse duration during the tetanus). These conditions are close to those in the experiments of Frey *et al.* (18), who recently reported that L-LTP appears to be normal in t-PA$^{-/-}$ mice. The higher temperature condition also more closely approximates the *in vivo* situation where t-PA mRNA increased 1 h after LTP-inducing tetanization (8). Even with higher temperature and stronger tetanization, a significant difference still remained between t-PA$^{-/-}$ and wild-type mice 3.5 h after tetanization (n = 4 each, $p < 0.001$) (Fig. 2*D*), although the onset of the decay in the mutant mice was now delayed to 2.5–3 h rather than 1–1.5 h.

Potentiation by a cAMP Analog or a D1/D5 Agonist Is Defective in t-PA$^{-/-}$ Mice. The t-PA gene has a cAMP-responsive element (CRE) in its promoter, which responds to cAMP and protein kinase A (19, 20). Brief application of the membrane permeable cAMP analog, Sp-cAMPS, to rat hippocampal slices produces a slowly developing, long-lasting potentiation that shares common mechanisms with L-LTP (1, 2). We therefore asked: Will t-PA$^{-/-}$ mice with a defect in L-LTP also have a defect in Sp-cAMPS-induced potentiation? To address this question, we applied Sp-cAMPS (100 μM, 15 min) to hippocampal slices and found that whereas the cAMP agonist produced a long-lasting potentiation in wild-type mice (154 ± 17% at 5 h, n = 6), it produced only a slight depression and no potentiation in t-PA$^{-/-}$ mice (85 ± 21% at 5 h, n = 6) (Fig. 3*A*). u-PA$^{-/-}$ mice had a normal response to Sp-cAMPS (Fig. 3*B*). These results provide further evidence for a selective defect in L-LTP in t-PA$^{-/-}$ mice and suggest that t-PA is one of the target genes of CREB activation.

Selective activation of the D1/D5 receptor, a seven transmembrane dopaminergic receptor coupled to adenyl cyclase, can stimulate L-LTP in the CA1 region of the hippocampus (21). This D1/D5 receptor agonist-induced potentiation is occluded by Sp-cAMPS potentiation and by the late phase of tetanus-induced potentiation. Bath application of the D1/D5 agonist 6-Br-APB hydrobromide (50 μM, 15 min) induced a long-lasting synaptic potentiation in wild-type mice (167 ± 20% at 3 h, n = 5), but not in t-PA$^{-/-}$ mice (103 ± 6%, n = 5) (Fig. 3*C*). This defect in the response to the D1/D5 agonist in t-PA$^{-/-}$ mice is consistent with the defect in Sp-cAMPS-induced and tetanus-induced L-LTP. These results suggest that during tetanus-induced L-LTP, activation of a dopaminergic pathway stimulates D1/D5 receptors, which increase cAMP, activate protein kinase A, and induce t-PA.

t-PA$^{-/-}$ Mice Also Have a Defect of L-LTP in the Mossy Fiber Pathway. A protein and mRNA synthesis-dependent L-LTP has also been found in the mossy fiber-CA3 pathway (14). In this pathway, we again found no difference in paired-pulse facilitation between wild-type and t-PA$^{-/-}$ mice (Fig. 4*A*). However, four trains of tetanization (100 Hz for 1 s, with double-pulse duration), which induced stable L-LTP in the mossy fiber-CA3 pathway of wild-type mice, induced a decaying L-LTP in t-PA$^{-/-}$ mice. Three hours after tetanus, L-LTP in t-PA$^{-/-}$ mice (118 ± 6%, n = 6) was significantly smaller than in wild-type mice (181 ± 8%, n = 6, $P < 0.01$, Student's t test) (Fig. 4*B*). Thus, in t-PA$^{-/-}$ mice, L-LTP is defective in both the mossy fiber-CA3 and the Schaffer collateral-CA1 pathways. This is of particular interest, because E-LTP in these pathways involves completely different mechanisms. Schaffer collateral E-LTP involves activation of NMDA receptors and a postsynaptic mechanism of induction. By contrast, induction of mossy fiber E-LTP is not dependent on NMDA

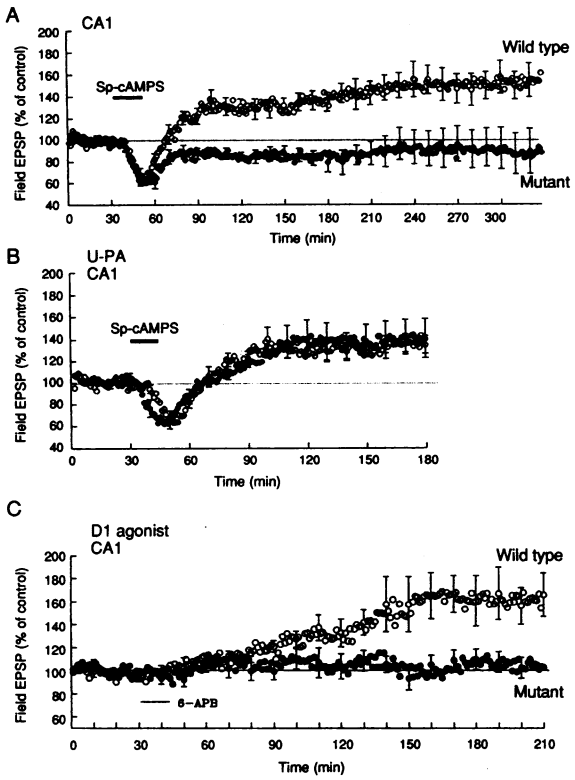

FIG. 3. cAMP analog- and D1 agonist-induced potentiation in the CA1 region of wild-type and t-PA$^{-/-}$ mice. (*A*) Brief application of Sp-cAMPS (100 μM, 15 min) induced a transient depression followed by a long-lasting potentiation of the field EPSP in wild-type mice (open circles). However, Sp-cAMPS induced no potentiation in t-PA$^{-/-}$ mice (closed circles) (n = 6 each). (*B*) Brief application of Sp-cAMPS-induced long-lasting potentiation in both wild-type and u-PA$^{-/-}$ mice (n = 4 each). (*C*) Brief application of the D1 agonist 6-Br-APB hydrobromide (50 μM, 15 min) induced a slowly developing long-lasting potentiation in wild-type mice (n = 5). However, no potentiation was induced by the application of 6-Br-APB hydrobromide in t-PA$^{-/-}$ mice (closed circles, n = 5).

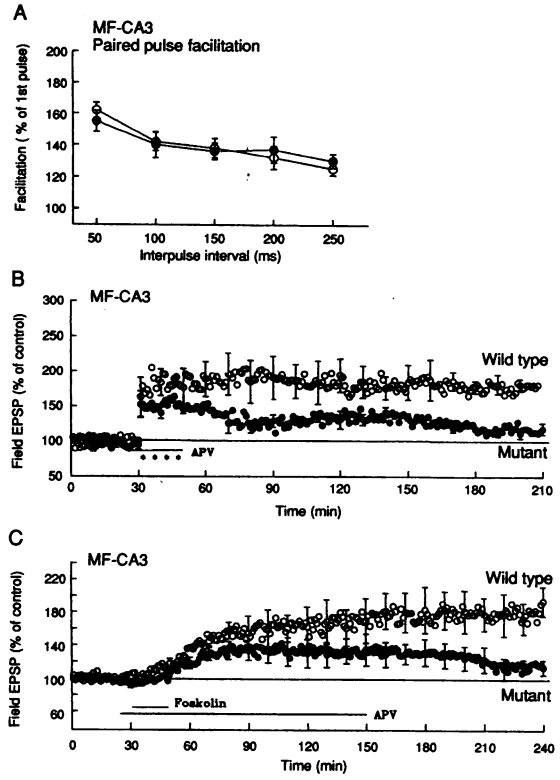

FIG. 4. Changes of synaptic plasticity in the mossy fiber-CA3 pathway of t-PA$^{-/-}$ mice. (*A*) There was no difference in paired-pulse facilitation between t-PA$^{-/-}$ and wild-type mice (n = 5 each). (*B*) Four trains of tetanus (100 Hz for 1 s, 0.1-ms pulse duration at 5-min intervals) induced a decaying LTP in t-PA$^{-/-}$ mice that was significantly different from that induced in wild-type mice 3 h after tetanus. 2-Amino-5-phosphonopentanoic acid (APV; 25 μM) was present during the tetanus in all experiments. (*C*). Brief application of forskolin (50 μM, 15 min) induced a long-lasting potentiation in wild-type mice (open circles) in the presence of 25 μM 2-amino-5-phosphonopentanoic acid (APV). By contrast, forskolin induced a slowly decaying potentiation in t-PA$^{-/-}$ mice (closed circles). The decay started about 1 h after drug application and became significantly different from that seen in wild-type mice 3 h after drug application (n = 5 each, $p < 0.01$).

receptors, is pre- rather than postsynaptic and requires a cAMP-dependent mechanism (14, 22).

The cAMP agonist forskolin can induce a macromolecular synthesis-dependent long-lasting potentiation in the mossy fiber-CA3 pathway, which shares a common step with tetanus-induced L-LTP (14). Forskolin induced long-lasting potentiation in wild-type mice, but in t-PA$^{-/-}$ mice it induced normal E-LTP but only a reduced L-LTP. In t-PA$^{-/-}$ mice, potentiation started to decay about 90 min after application of forskolin; by 3 h after tetanus, the potentiation in t-PA$^{-/-}$ mice was significantly different from forskolin-induced potentiation in wild-type mice (114 ± 9% and 193 ± 18%, respectively, n = 5 each, $p < 0.01$) (Fig. 4*C*). These results in the mossy fiber pathway parallel the defect in Sp-cAMPS-induced potentiation in the Schaffer collateral-CA1 pathway of t-PA$^{-/-}$ mice, indicating that in both pathways, t-PA is one of the downstream target proteins of the cAMP cascade. t-PA thus appears to be critical for the late phase of both NMDA-dependent and NMDA-independent LTP.

t-PA$^{-/-}$ Mice Are Not Impaired in Novel Exploration, Context Conditioning, and Spatial Memory. Rodents with hippocampal lesions exhibit a significant increase in activity (23) in a novel environment, which may be due to a disruption in the ability to gather the spatial information necessary to establish an internal representation of the environment (24). We examined the activity levels of t-PA$^{-/-}$ and wild-type mice and found that there was no significant effect of genotype, although there was a significant effect of gender (Fig. 5*A*). These results suggest that the mutants do not have a deficit in gathering spatial information and also do not have severe sensory, attentional, or motor defects.

To assess more explicitly spatial (place) recognition, we next examined performance on another hippocampus-dependent task, context conditioning, which occurs when a novel environment (context) is paired with an aversive stimulus (a footshock) (25, 26). If learning occurs, the novel environment comes to elicit fear, as evidenced by freezing (lack of movement) in the mice. We also examined a variation of this task that is not hippocampus-dependent but is amygdala-dependent, cued conditioning. In cued conditioning, a discrete neutral stimulus (a tone) is paired with the footshock. As indicated by the amount of time spent freezing on the training and testing days, t-PA$^{-/-}$ and wild-type mice acquired both the tone-shock and context-shock associations (Fig. 5 *B1* and *B2*). Surprisingly, female t-PA$^{-/-}$ mice froze significantly more to tone on the training day and to both tone and context on the testing day. This result might be due to either enhanced performance (generalized freezing) or enhanced learning in the female t-PA$^{-/-}$ mice, but in either case it does not suggest a deficit in learning. Thus, both male and female t-PA$^{-/-}$ mice were evidently able to form an internal representation of the context that they used for spatial recognition on the testing day, and they were also able to learn a simple association between a tone and footshock. These results suggest that ablation of the t-PA gene did not severely disrupt functioning of the hippocampus or amygdala during learning.

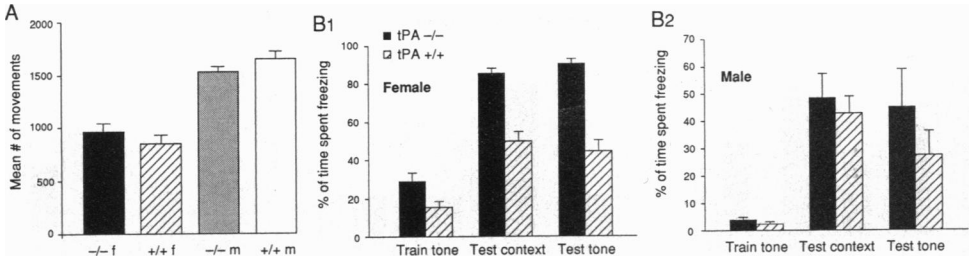

FIG. 5. Tests of novel exploration and conditioned fear. (*A*) Amount of exploration by male and female t-PA$^{-/-}$ and wild-type mice in a novel environment. Values represent group means ± SEM. A two-factor ANOVA revealed no significant effect of genotype, but there was a significant effect of gender [$F(1,19) = 93.169, p < 0.0001$]. (*B*) Percentage of time spent freezing to tone during training and freezing to tone and context during testing by t-PA$^{-/-}$ and wild-type mice. (*B1*) Females. (*B2*) Males. Values represent group means ± SEM. A two-factor ANOVA (genotype and gender) was calculated for each dependent variable: freezing to tone during training [genotype, $F(1,20) = 7.145, p < 0.05$; gender, $F(1,20) = 44.329, p < 0.001$; and genotype and gender, $F(1,20) = 6.003, p < 0.05$], freezing to context during testing [genotype, $F(1,20) = 11.293, p < 0.005$; gender, $F(1,20) = 12.983, p < 0.005$; and genotype and gender, $F(1,20) = 6.003, p < 0.05$], and freezing to tone during testing [genotype, $F(1,20) = 12.979, p < 0.005$; and gender, $F(1,20) = 12.752, p < 0.005$].

The ability to form an internal representation of an environment can be dissociated from the ability to use that representation to navigate to biologically significant areas within the environment (24, 27, 28). Both functions are thought to be hippocampus-dependent but may not be mediated by the same hippocampal regions or molecular mechanisms. We therefore examined the spatial navigation ability of t-PA$^{-/-}$ and wild-type mice using both the Barnes circular and Morris water mazes. The majority of mice (5 out of 6) in both the t-PA$^{-/-}$ and wild-type groups reached criterion on the Barnes maze. Furthermore, the number of days to criterion was not significantly different for both groups (wild-type median = 28, mutant median = 30) (Fig. 6*A*), and there was no significant difference between groups in the number of errors across session blocks (Fig. 6*B*). The t-PA$^{-/-}$ and wild-type mice were also not significantly different in the accuracy of their initial search across session blocks (Fig. 6*C*). These results suggest that both wild-type and t-PA$^{-/-}$ mice were able to remember and use the relationship between the distal cues (e.g., use a spatial strategy) to navigate to the escape tunnel.

In the Morris maze, the t-PA$^{-/-}$ mice did have significantly longer swim paths during both acquisition learning (days 1–3) and reversal learning (days 4 and 5) (Fig. 7*A1*). Longer swim paths might be due to either a deficit in spatial memory or some performance factor such as a motivational, sensory, or motor deficit. Computer analysis of swim paths (29) did not reveal any gross motor anomalies in terms of swimming speed or path tortuosity, indicating that the longer swim paths were not due to a gross motor deficit (data not shown). However, the longer swim paths of the t-PA$^{-/-}$ mice were partially related to thigmotaxis, an increased tendency to swim close to the wall of the pool ($r = -0.44, p = 0.0002, n = 68$; data not shown). Furthermore, during the first trial of reversal learning, t-PA$^{-/-}$ mice crossed the old platform location as frequently as the wild-type mice (Fig. 7*A2*), indicating that they were able to use a spatial strategy to navigate to the location of the platform. These results suggest that spatial learning was not defective in the t-PA$^{-/-}$ mice, and that their increased swim paths were therefore due to some other deficit.

t-PA$^{-/-}$ Mice Are Impaired on Two-Way Active Avoidance. To further characterize the behavioral deficit of the t-PA$^{-/-}$ mice, we tested most of the mice that had been used in the Morris water maze on two-way active avoidance in a shuttlebox. In this task, the mice could avoid a footshock, signaled by a stimulus light, by running into the second chamber of the shuttlebox. Accurate active avoidance performance is not hippocampus-dependent, but is thought to be striatum-dependent (30–32). The t-PA$^{-/-}$ mice were significantly worse at avoiding the footshock, particularly during the last 3 days of testing (Fig. 7*B*). We observed no evidence for performance deficits such as reduced footshock sensitivity, motor impairment, or abnormal freezing in any of the mice tested in this task. However, poor active avoidance was significantly correlated with the degree of thigmotaxis in the Morris maze ($r = -0.42, p = 0.0008, n = 60$). The deficit in active avoidance and thigmotaxis in the Morris maze could both result from an inability to learn the correct motor response, perhaps as a result of striatal disruption. Alternatively, they could both be due to subtle performance deficits we were not able to detect.

DISCUSSION

Our experiments provide a genetic dissection of LTP, which indicates that in the Schaffer collateral and the mossy fiber pathways, LTP has early and late phases that are distinguishable by their requirement for t-PA. These point to t-PA as a critical downstream effector gene for L-LTP and are consistent with the finding that LTP protocols lead to the induction of t-PA. Moreover, the finding that ablation of t-PA selectively blocks L-LTP and not E-LTP is consistent with pharmacological studies suggesting that L-LTP in both the Schaffer collateral and mossy fiber pathways requires new protein and mRNA synthesis and that in both of these pathways the late phase involves cAMP and protein kinase A (1–3). Furthermore, we find that in the absence of t-PA, cAMP analogs or adenylate cyclase activators cannot induce L-LTP in either the Schaffer collateral or mossy fiber pathways. This result is consistent with the finding that the t-PA gene has a CRE element in its promoter and suggests that t-PA is an

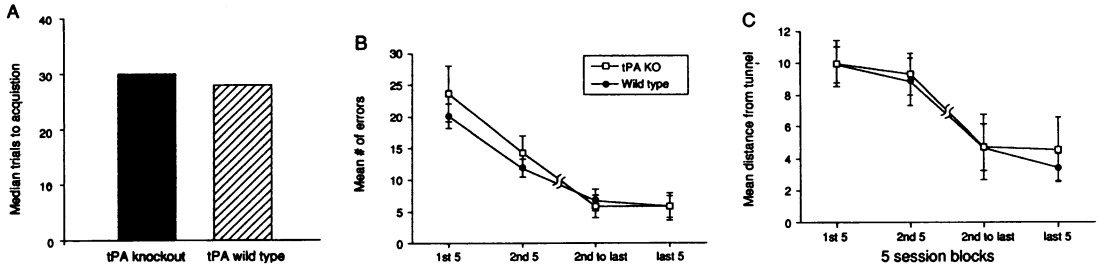

FIG. 6. Performance of t-PA$^{-/-}$ and wild-type mice on the Barnes circular maze. (*A*) Median trials to acquisition of t-PA$^{-/-}$ and wild-type mice. (*B*) Errors. (*C*) Distance between the first hole searched and the escape tunnel. Values for errors and distance represent group means ± SEM across blocks of five sessions.

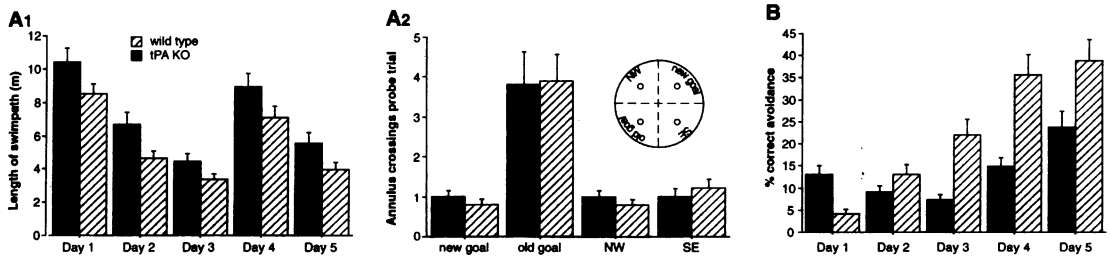

FIG. 7. Performance of t-PA$^{-/-}$ and wild-type mice on the Morris water maze and two-way active avoidance. (*A*) Morris water maze. (*A1*) Acquisition on days 1–3 and reversal learning after platform relocation on days 4–5. Data were analyzed by a two-way ANOVA with one repeated measure. A significant effect was obtained for genotype [$F(1,66) = 8.68, p < 0.005$] and day [$F(1,66) = 39.478, p < 0.0001$]. Values represent group means ± SEM. (*A2*) Number of annulus crossings in four quadrants during the first trial of reversal learning ("probe trial"). No significant effects of genotype were revealed by a two-way ANOVA, with both t-PA$^{-/-}$ and wild-type mice crossing the former platform location significantly more often than the analogous locations in the other quadrants [$F(1,66) = 26.8, p < 0.0001$]. (*B*) Performance of t-PA$^{-/-}$ and wild type mice in two-way active avoidance. The percentage of successful avoidances of foot shock are represented by group means ± SEM. A two-factor ANOVA with one repeated measure revealed a significant effect of genotype [$F(1,58) = 8.66, p < 0.005$].

important downstream target of cAMP. The critical role of t-PA in L-LTP is also supported by the recent finding that inhibitors of t-PA blocked L-LTP selectively and that the application of recombinant t-PA induces L-LTP in rat hippocampal slices (17).

After these studies were completed, Frey *et al.* (18) reported that L-LTP in t-PA$^{-/-}$ mice was deficient in the presence of the γ-aminobutyric acid antagonist picrotoxin, but appeared to be normal in normal saline. By contrast, we found a deficit in L-LTP in t-PA$^{-/-}$ mice in the absence and presence of picrotoxin. The difference between these two results may stem from differences in the extent of tonic inhibition due to γ-aminobutyric acid-ergic interneurons in the different experimental conditions, since tonic inhibition by γ-aminobutyric acid-ergic interneurons in hippocampal slices is influenced by many factors, such as temperature and stimulation intensity. Consistent with this possibility, we found that the deficit in L-LTP in t-PA$^{-/-}$ mice had a slower onset at a higher temperature and tetanus intensity (Fig. 2*D*).

What is the function of t-PA in the hippocampus? Stimuli that evoke LTP are capable of inducing structural changes, but how this occurs is not known. Perhaps t-PA-mediated extracellular proteolysis activates a ligand that engages a receptor essential for the structural remodeling (33, 34). t-PA is released from growth cones, and this release is correlated with morphological differentiation and induction (12, 16). In addition, t-PA seems also to be stored in the microglia of the hippocampus (11). The release of t-PA from neurons or microglia could contribute to the initiation of activity-dependent structural changes in one of several ways: (*i*) by altering adhesive contacts between neurons (35, 36), (*ii*) by changing the spatial and temporal interactions between proteases and their inhibitors, and (*iii*) by activating a ligand that could bind a receptor in a manner similar to that activated by thrombin (37).

Our behavioral experiments indicate that although disruption of the t-PA gene produced a deficit in L-LTP in the hippocampus, it did not affect performance on the hippocampus-dependent behavioral tasks that we employed. This contrasts with findings from mice lacking isoforms of CREB, which are also deficient in L-LTP but do show a major defect in hippocampus-dependent long-term memory tasks (38). The lack of a significant hippocampal-dependent behavioral deficit in the t-PA$^{-/-}$ mice despite a defect in L-LTP might be related to two factors. First, since temperature can compensate for aspects of the severity of this defect, it is possible that in the intact behaving animal this defect could be much smaller, as suggested by the results shown in Fig. 2*D* and those of Frey *et al.* (18). Second, unlike the CREB knockout mice, where the defect is reflected in a complete abolition of the late phase, L-LTP is not fully absent in t-PA$^{-/-}$ mice. Perhaps L-LTP needs to be decreased below a certain threshold before a behavioral deficit emerges. If this is so, genetic experiments that interfere with the late phase of L-LTP in a graded and progressive way may reveal the degree of deficit necessary to interfere with hippocampus-based long-term memory storage.

We thank Ted Abel and Steve Siegelbaum for critically reading this manuscript. We thank C. Lam for helping with the figures, H. Ayers and I. Trumpet for typing the manuscript, and Dr. M. Osman for veterinary care of the mice. This work was supported by the Howard Hughes Medical Institute and Grant MH50733 from the National Institute of Mental Health to E.R.K. as well as the Columbia component of the Dana Consortium on Age-Related Memory Loss (Y.-Y.H.).

1. Frey, U., Huang, Y.-Y. & Kandel, E. R. (1993) *Science* **260,** 1661–1664.
2. Huang, Y.-Y. & Kandel, E. R. (1994) *Learn. Mem.* **1,** 74–82.
3. Nguyen, P. V., Abel, T. & Kandel, E. R. (1994) *Science* **265,** 1104–1107.
4. Scoville. W. B. & Milner, B. (1957) *Neurosurg. Psychol.* **20,** 11–21.
5. Squire, L. R. (1992) *Psychol. Rev.* **99,** 195–231.
6. Bailey, C. H. & Kandel, E. R. (1993) *Annu. Rev. Physiol.* **55,** 397426.
7. Greenough, W. T. (1984) *Trends Neurosci.* **7,** 229–233.
8. Qian. Z., Gilbert, M. E., Colicos, M., Kandel, E. R. & Kuhi, D. (1993) *Nature (London)* **361,** 453–457.
9. Colien, D. & Lijnen, H. R. (1991) *Blood* **78,** 3114–3124.
10. Sappino, A. P., Madani, R. Huarte, J., Belin, D., Kiss, J. Z., Wohlwend, A. & Vassali, J. D. (1993) *J. Clin. Invest.* **92,** 679–685.
11. Tsirka, S. E., Gualandris, A., Amaral, D. G. & Strickland, S. (1995) *Nature (London)* **28,** 340–344.
12. Krystosek, A. & Seeds, N. W. (1981) *Science* **213,** 1532–1534.
13. Carmeliet, P., Schoonjans, L., Kieckens, L., Ream, B., Degen, I., Bronson, R., De Vos, R., van den Oord, J. J., Collen, D. & Mulligan, R. C. (1994) *Nature (London)* **368,** 419–424.
14. Huang. Y.-Y., Li. X.-C. & Kandel. E. R. (1994) *Cell* **79,** 69–79.
15. Schöpke, R., Wolfer, D. P., Lipp, H. P. & Leisinger-Trigona, M. O. (1991) *Hippocampus* **1,** 315–328.
16. LeVay, S., Hubel, D. H. & Wiesel, T. N. J. (1975) *Comp. Neurol.* **159,** 559–576.
17. Huang, Y.-Y., Abel, T. & Kandel, E. R. (1995) *Soc. Neurosci. Abstr.* **21,** 712.10.
18. Frey, U., Müller, M. & Kuhl, D. (1996) *J. Neurosci.* **16,** 2057–2063.
19. Saksela, O. & Rifkin, D. B. (1988) *Annu. Rev. Cell Biol.* **4,** 93–126.
20. Baranes, D., Martin, K. & Lederfein, D. (1995) *Soc. Neurosci. Abstr.* **21,** 712.9.
21. Huang, Y.-Y. & Kandel, E. R. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 2446–2450.
22. Weisskopf, M. G., Castillo, P. E., Zalutsky, R. A. & Nicoll, R. A. (1994) *Science* **265,** 1878–1882.
23. Myhner, T. (1988) *Behav. Neurosci.* **102,** 356–362.
24. Poucet, B. (1993) *Psychol. Rev.* **100,** 163–182.
25. Kim, J. J. & Fanselow, M. S. (1992) *Science* **256,** 675–677.
26. Phillips, R. G. & LeDoux, J. E. (1992) *Behav. Neurosci.* **106,** 274–285.
27. Müller, R. U., Kubie, J. L., Bostock, E. M., Staube, J. S. & Quirk, G. J. (1991) *Brain and Space* (Oxford Univ. Press, New York), pp. 297–333.
28. Bach, M. E., Hawkins, R. D., Osman, M., Kandel, E. R. & Mayford, M. (1995) *Cell* **81,** 905–915.
29. Wolfer, D. P. & Lipp, H.-P. (1992) *J. Neurosci. Methods* **41,** 65–74.
30. Green, R. H., Beatty, W. W. & Schwartzbaum, J. S. (1967) *J. Comp. Physiol. Psychol.* **64,** 444–452.
31. McDonald, R. J. & White, N. M. (1993) *Behav. Neurosci.* **107,** 3–22.
32. Winocur, G. J. (1974) *Comp. Physiol. Psychol.* **86,** 432–439.
33. Moonen, G., Grau-Wagemans, M. P. & Selak, I. (1982) *Nature (London)* **298,** 753–755.
34. Quigley, J. P., Gold, L. I., Schwimmer, R. & Sullivan, L. M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2776–2780.
35. Pittman, R. N., Ivins, J. K. & Buettner, H. M. (1989) *J. Neurosci.* **9,** 42694286.
36. Ben-Ari, T. & Represa, A. (1990) *Trends Neurosci.* **13,** 312–318.
37. Hajark, K. A. (1991) *J. Biol. Chem.* **266,** 21962–21970.
38. Bourtchuladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schuta, G. & Silva, A. J. (1994) *Cell* **79,** 59–68.