

Transgenic Mice Lacking NMDAR-Dependent LTD Exhibit Deficits in Behavioral Flexibility

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SUMMARY

While most studies have focused on the role of long-term potentiation in behavior, far less is known about the role of long-term depression (LTD). To examine the potential involvement of LTD in learning and memory, we generated transgenic mice that express a fragment of the SV40 small t antigen known to inhibit protein phosphatase 2A (PP2A). Small t antigen expression blocked both stimulus-induced and chemically induced NMDAR-dependent LTD at Schaffer collateral synapses but did not affect potentiation, depotentiation, or mGluR-dependent LTD. This physiological phenotype was associated with deficits in behavioral flexibility in both the Morris water maze and a delayed nonmatch to place T-maze task, suggesting that NMDAR-dependent LTD is required for behavioral flexibility and may act by weakening previously encoded memory traces when new information is learned.

INTRODUCTION

The involvement of the hippocampus in declarative memory is well established, and many studies suggest that changes in synaptic plasticity play a central role in this function. Most models of memory storage, based on changes in synaptic efficacy, require that those changes occur bidirectionally to protect the network from the saturating effects of potentiation alone (Martin and Morris, 2002; Rosenzweig et al., 2002). In theory, this saturation can occur at two levels, at the cellular level and at the network level. As a consequence of the inherent physical limitations of the cell, unchecked potentiation of synapses in an individual neuron could prevent it from participating in the storage of additional information, and it is thought that neurons counter this tendency through a process known as synaptic scaling (Burrone and Murthy, 2003; Turrigiano and Nelson, 2004). A second type of saturation could occur at the network level wherein potentiated synapses that constitute earlier memory traces interfere

with subsequent memory traces stored in distinct or overlapping sets of synapses. This possibility has led to the proposal that decreases in synaptic efficacy may serve to increase the “signal to noise ratio” between the potentiated synapses that participate in a memory trace and the nonpotentiated synapses that do not. The suggestion that persistent decreases in synaptic efficacy (LTD) may perform this function comes from three observations. First, exposure of animals to a novel environment reverses long-term potentiation induced in vivo (Xu et al., 1998). Second, exposure to novel spatial information enhances LTD induction (Kemp and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 1999). Third, pharmacological blockade of NMDA receptor-dependent forms of plasticity, which include synaptic depression, both preserve in vivo potentiation and increase retention of spatial memory (Villarreal et al., 2002).

One complicating factor in understanding the role of synaptic depression in the hippocampus is the presence of multiple forms of LTD (Anwyl, 2006). In rodents older than 3 weeks, two distinct forms of LTD coexist at Schaffer collateral synapses in the hippocampus. The first requires the activity of NMDA receptors and the second the activity of metabotropic glutamate receptors (mGluRs), specifically mGluR5 (Huber et al., 2001, but see Volk et al., 2006). Although internalization of AMPA receptors occurs in both cases, the two forms are not mutually occluding, and the pathways leading to receptor internalization appear to be largely distinct (Carroll et al., 1999; Oliet et al., 1997; Snyder et al., 2001). One difference is in the requirement for serine/threonine phosphatase activity. The NMDAR-dependent form of LTD exhibits a requirement for serine/threonine phosphatases while the mGluR-dependent form does not (Schnabel et al., 2001; Winder and Sweatt, 2001).

Given its selective requirement for serine/threonine phosphatase activity, we attempted to interfere with the NMDAR-dependent form of LTD and assess its possible contribution to normal behavior by targeting the enzyme PP2A. PP2A is a highly abundant serine/threonine phosphatase composed of three subunits: a 36 kDa catalytic C subunit, a 65 kDa structural A subunit, and a regulatory B subunit. Multiple isoforms exist for each of these three subunits, and different combinations of these isoforms give rise to a wide variety of PP2A enzymes with different subcellular distributions and substrate specificities (Janssens and

Goris, 2001; Zolnierowicz, 2000). A well-known and extensively characterized inhibitor of PP2A is the small t antigen of simian virus 40 (SV40) (Chen et al., 2004; Mumby, 1995). The SV40 small t antigen protein consists of two domains, an N-terminal region, common to both the large and small t antigens, and a C-terminal region, unique to small t, that is necessary and sufficient for PP2A inhibition (Sontag et al., 1993). This unique region domain associates with PP2A via binding sites on the A subunit that overlap with sites responsible for binding B subunits (Ruediger et al., 1992). Small t therefore competes with endogenous B subunits for binding to the core PP2A dimer and is thought to do so more effectively with some B subunits than others (Chen et al., 2004; Van Hoof and Goris, 2004).

To better understand the relationship between LTD and behavior, we physiologically and behaviorally characterized transgenic mice that expressed the PP2A-inhibitory portion of small t antigen in principal cells of the forebrain. We found that expression of small t blocked both stimulus-induced and chemically induced NMDAR-dependent LTD, while sparing potentiation, depotentiation, and mGluR-dependent LTD. Analysis of the effects of small t expression in cultured hippocampal neurons showed that it also inhibited NMDA-induced AMPA receptor internalization without affecting internalization induced by mGluR activation. This specific deficit in NMDAR-dependent LTD allowed us to examine the contribution of this form of plasticity to behavior in the absence of deficits in other forms of plasticity. We found that the NMDAR-dependent LTD deficit was associated with deficits in behavioral flexibility in two different behavioral tasks. Furthermore, by temporally regulating small t expression we found that the physiological and the behavioral deficits were dependent on the contemporaneous expression of the transgene.

RESULTS

Small t Antigen Transgene and Expression

To inhibit PP2A activity *in vivo*, we generated a transgene encoding the C-terminal 86 amino acids of the SV40 small t antigen unique region responsible for binding PP2A (Figure 1A). We affixed a hemagglutinin epitope tag to the N terminus of the truncated protein and confirmed its ability to bind PP2A A/C core dimers by immunoprecipitating complexes containing small t antigen, A, and C subunits as previously reported (Figure 1B; Mateer et al., 1998; Sontag et al., 1993). We placed this construct under the control of a synthetic tetO promoter and crossed animals carrying this transgene to animals carrying a transgene where expression of the synthetic transactivator, tTA, was driven by the α -calcium/calmodulin kinase II promoter (*CaMKII*) (Mayford et al., 1996). In double-transgenic progeny that carried both the *tetO-small t* and *CaMKII α -tTA* transgenes, tTA bound to the *tetO* promoter and activated small t expression only in principal cells of the forebrain where the *CaMKII α* promoter was active (see Figure 2C). Oligonucleotide *in situ* hybridization to sections from *tetO-small t/CaMKII α -tTA* double-transgenic animals revealed expression of small t RNA throughout the forebrain including the cerebral cortex, olfactory bulb, striatum, and hippocampus, with particularly strong expression in the hippocampal CA1 pyramidal cell layer (Figures 2A and 2B). This

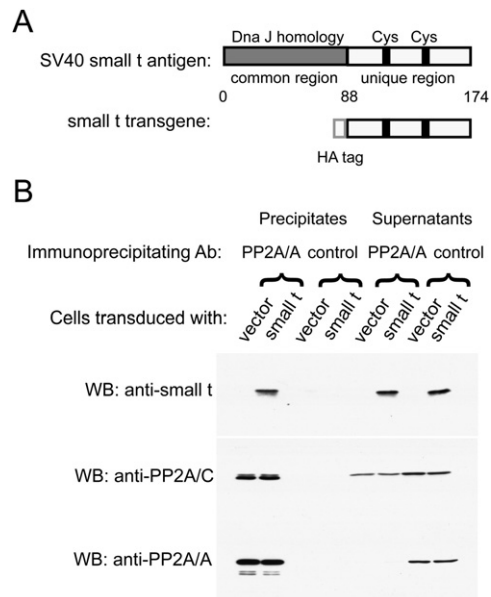


Figure 1. The SV40 Small t Antigen Transgene Binds to PP2A

(A) Schematic representation of the SV40 small t antigen transgene.

(B) Western blots, using the indicated primary antibodies, on immunoprecipitates and supernatants prepared from virally transduced cells using either an anti-PP2A/A subunit antibody or control antibody.

pattern of expression was present in both juvenile and adult animals, while no expression was observed in single-transgenic controls (data not shown). Small t expression did not perturb hippocampal anatomy as assessed by Nissl staining (Figures 2A and 2B) or by the immunohistochemical markers GluR1, Map2, syntaxin, and GFAP (see Figure S1A available online).

Binding of the tTA transactivator to the *tetO* promoter is inhibited by tetracycline or its analogs (Mayford et al., 1996). We could therefore temporally control small t antigen expression by administering doxycycline in the animals' food. To inhibit small t expression in 3- to 4-week-old juvenile animals, we maintained pregnant females and their litters on food containing 40 mg/kg doxycycline. This prevented the expression of both small t RNA (Figure 2A) and protein (Figures 2D and S1B) in *tetO-small t/CaMKII α -tTA* double-transgenic animals. To selectively inhibit small t expression in adult double-transgenic animals, we maintained animals on food without doxycycline until 6 weeks of age, at which point we switched the animals to doxycycline-containing food. This shift resulted in near complete inhibition of small t RNA expression in 3-month-old adults, while preserving expression at earlier stages (Figure 2B).

Small t Expression Does Not Affect Basal Synaptic Transmission (I/O or PPF)

To determine if small t antigen expression affected basal synaptic transmission at Schaffer collateral synapses, we assessed the input/output relationship at different stimulus intensities and examined paired-pulse facilitation at different interstimulus intervals. We found no significant differences in the input/output relationship for slices prepared from juvenile or adult small

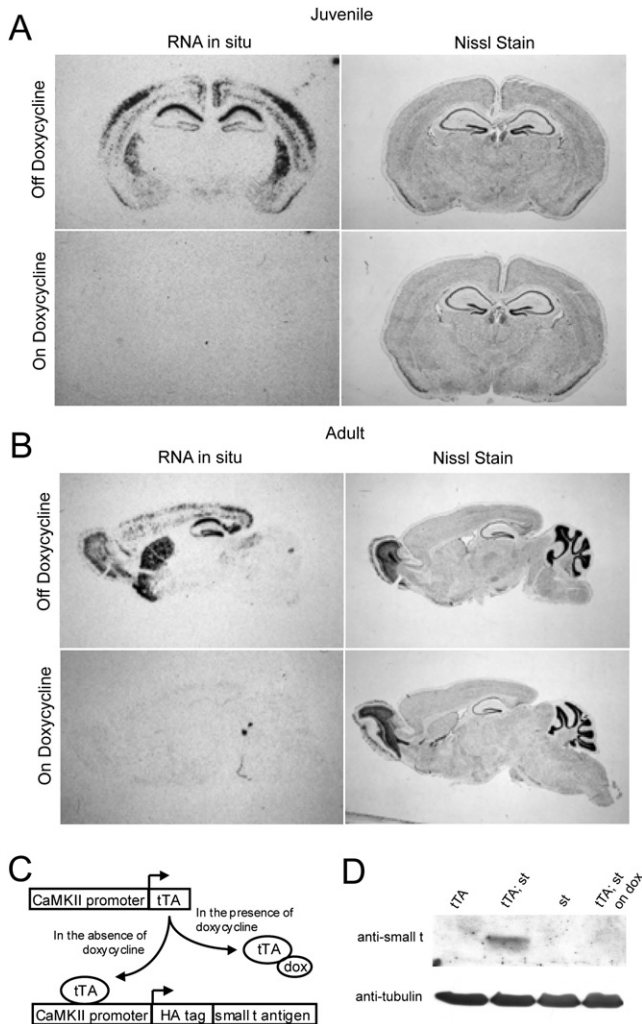


Figure 2. Doxycycline-Regulated Expression of Small t Antigen in the Forebrain of Double-Transgenic Mice

(A) Oligonucleotide in situ hybridization to small t antigen RNA in coronal brain sections from 21- to 28-day-old double-transgenic animals raised either off doxycycline (upper left) or on doxycycline (lower left), and subsequent Nissl stains of the same sections (right panels).

(B) Oligonucleotide in situ hybridization to small t antigen RNA in sagittal brain sections from 3-month-old double-transgenic animals raised either off doxycycline (upper left) or shifted onto doxycycline-containing food beginning at 6 weeks of age (lower left), and subsequent Nissl stains of the same sections (right panels).

(C) Schematic representation of the transgenes used to achieve doxycycline-regulated forebrain-restricted expression of SV40 small t antigen.

(D) Western blot showing small t antigen protein present in hippocampal homogenates from 21- to 28-day-old double-transgenic animals (tTA; st) but not single-transgenic sibling controls (tTA or st alone) or double-transgenic controls raised on doxycycline.

t-expressing double-transgenic animals and single-transgenic controls (henceforth controls), indicating that small t expression did not affect this aspect of basal synaptic transmission (Figure 3A; repeated-measures ANOVA for effect of genotype, $p = 0.16$ from $n = 13/8, 14/8$ slices/animals for double transgenics and controls, respectively; see also Figure S2A). Neither

was paired-pulse facilitation (PPF) examined at different inter-stimulus intervals affected by small t expression (Figure 3B; repeated-measures ANOVA for effect of genotype, $p = 0.20$, $n = 10/5, 10/5$).

Small t Expression Blocks LTD at Schaffer Collateral Synapses

LTD is known to depend on serine/threonine protein phosphatase activity (Winder and Sweatt, 2001). Consistent with this, we found that bath application of fostriecin at concentrations selective for PP2A ($0.14 \mu\text{M}$) inhibited stimulus-induced LTD at Schaffer collateral synapses in hippocampal slice preparations (Figure S1C). We therefore tested the ability of small t antigen to inhibit LTD at this synapse. In slices from 21- to 28-day-old controls, a 15 min, 1Hz stimulus train evoked persistent depression of evoked responses that averaged $50\% \pm 5\%$ of baseline at 40–60 min post-stimulus (Figure 3C, filled circles), which was absent in slices from double-transgenic animals (Figure 3C, gray squares; $93\% \pm 1\%$ of baseline at 40–60 min, $p = 0.0005$, $n = 17/17, 13/13$), and dependent on small t expression, since LTD was restored in double-transgenic slices by doxycycline administration (Figure 3D; $73\% \pm 7\%$ of baseline at 40–60 min for double transgenics versus $72\% \pm 8\%$ for controls, $p = 0.96$, $n = 7/5, 8/5$).

The magnitude of stimulus-induced LTD decreases with the age of the animal (Dudek and Bear, 1993); however, LTD that averaged $86\% \pm 3\%$ of baseline at 40–60 min post-stimulus could still be observed in control animals at 3 months of age (Figure 3E, filled circles). As with juveniles, we found that small t expression blocked LTD at this age (Figure 3E, gray squares; $99\% \pm 4\%$ of baseline at 40–60 min, $p = 0.04$, $n = 12/6, 12/6$). This blockade was dependent on contemporaneous expression of small t, since shifting the animals onto doxycycline-containing food at 6 weeks of age restored LTD (Figure 3F; $85\% \pm 13\%$ of baseline at 40–50 min for double transgenics versus $85\% \pm 11\%$ for controls, $p = 0.97$, $n = 9/5, 9/5$).

In addition to low-frequency stimulation, LTD can be induced chemically, by brief exposure of slices to NMDA (Lee et al., 1998). We found that a 5 min application of $30 \mu\text{M}$ NMDA to hippocampal slices (see Figure S2B) resulted in persistent depression of Schaffer collateral evoked responses in controls that was blocked in small t-expressing slices (Figure 3G; $100\% \pm 10\%$ of baseline at 100–120 min for double transgenics versus $46\% \pm 9\%$ for controls, $p = 0.0008$, $n = 14/8, 12/7$). Like the stimulus-induced form of LTD, this deficit in chemically induced LTD was also reversed by maintaining the double-transgenic animals on doxycycline-containing food (Figure 3H; $66\% \pm 8\%$ of baseline at 100–120 min for double transgenics versus $56\% \pm 5\%$ for controls, $p = 0.34$, $n = 8/4, 8/4$). Treatment with NMDA also caused persistent depression in slices from 3-month-old animals that was also blocked by small t expression (Figure 3I; $88\% \pm 9\%$ of baseline at 100–120 min for double transgenics versus $52\% \pm 6\%$ for controls, $p = 0.005$, $n = 10/6, 10/5$) and restored when these animals were shifted to doxycycline-containing food at 6 weeks of age (Figure 3J; $74\% \pm 7\%$ of baseline at 100–120 min for double transgenics versus $63\% \pm 9\%$ for controls, $p = 0.36$, $n = 8/5, 9/5$).

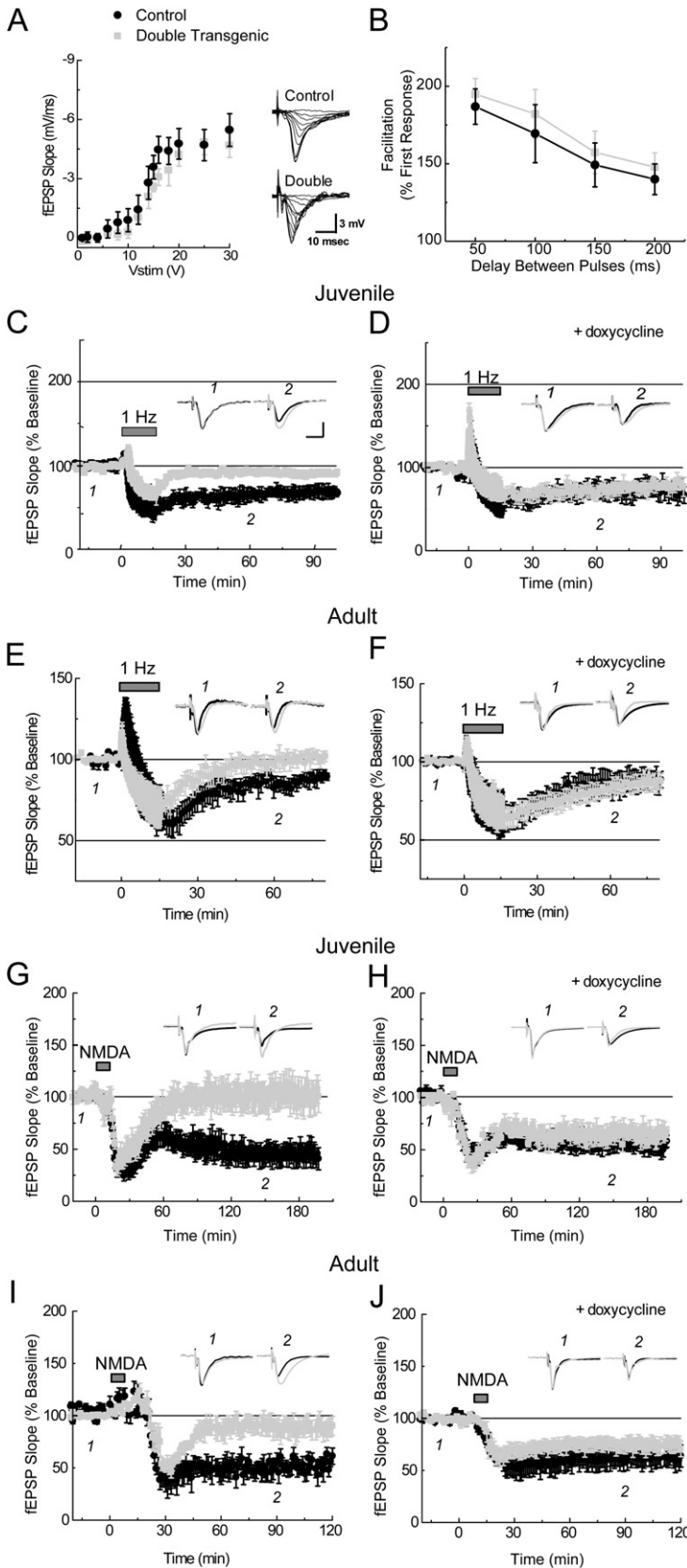


Figure 3. Basal Synaptic Transmission Is Normal, but LTD Is Absent in Small t-Expressing Mice

Averaged initial fEPSP slopes \pm SEM obtained at Schaffer collateral synapses in slices from 21- to 28-day-old animals (A–D, G, and H) or 3-month-old adults (E, F, I, and J). Insets show sample traces taken before (1) and after stimulation (2) for double-transgenic animals (gray) and controls (black).

(A) Input/output relationship and representative field EPSP sample traces.

(B) Paired-pulse facilitation at the indicated interstimulus intervals. (C–F) LTD induced by a 15 min, 1 Hz stimulus train.

(G–J) LTD induced by 5 min exposure to 30 μ M NMDA. Suppression of small t expression was achieved by raising animals on doxycycline-containing food (D and H) or shifting animals onto doxycycline-containing food at 6 weeks (F and J).

Small t Expression Does Not Affect LTP

Previous genetic manipulations that resulted in LTD deficits gave rise to corresponding enhancements in potentiation (Chen et al., 2003; Migaud et al., 1998; Zeng et al., 2001). This phenomenon is known as a metaplastic shift and is thought to reflect the fact that potentiation and depression result from opposing actions on common effector mechanisms. To determine if the small t-expressing animals exhibit such a shift, we measured the responses at Schaffer collateral synapses to a number of different potentiating stimulus protocols. In acute slices from 3- to 4-week-old animals, 4 × 1 s, 100 Hz stimulus trains elicited an enduring form of potentiation in both double transgenic and control slices, suggesting that small t does not affect this form of potentiation (Figure 4A; Table S2; 146% ± 11% of baseline at 100–120 min for double transgenics versus 159% ± 7% for controls, $p = 0.18$, $n = 6/6$, $6/6$). A single 1 s, 100 Hz train resulted in a short-lasting potentiation that is often larger in magnitude or longer in duration in animals that exhibit a metaplastic shift; however, we found no evidence for increases in either measure in animals that express small t (Figure 4B; Table S2; 108% ± 3% of baseline at 100–120 min for double transgenics versus 106% ± 2% for controls, $p = 0.47$, $n = 6/6$, $6/6$). A single 1 s train at 50 Hz also evokes short-term potentiation that is similarly unaffected by small t expression (Figure 4C; Table S2; 105% ± 6% of baseline at 100–120 min for double transgenics versus 98% ± 2% for controls, $p = 0.33$, $n = 5/5$, $5/5$). Stimulation for 90 s at 10 Hz resulted in potentiation that was initially greater in small t-expressing slices during the first 20 min following stimulation but was indistinguishable from controls beyond that point (Figure 4D; Table S2; 124% ± 5% of baseline at 100–120 min for double transgenics versus 127% ± 2% for controls, $p = 0.58$, $n = 8/6$, $8/6$). Five hertz stimulation for 3 min elicited depression that rapidly returned to baseline in both small t-expressing and control slices (Figure 4E; Table S2; 101% ± 3% of baseline at 1–20 min for double transgenics versus 98% ± 1% for controls, $p = 0.53$, $n = 5/5$, $5/5$). Finally, small t expression also did not affect responses to a single theta burst train of stimulation (9 bursts of 4 pulses at 100 Hz with 200 ms intervals; Figure 4F; Table S2; 148% ± 4% of baseline at 100–120 min for double transgenics versus 142% ± 11% for controls, $p = 0.63$, $7/5$, $7/5$).

The normal LTP that we observed in 3- to 4-week-old juvenile animals was also present in adults. Four 1 s, 100 Hz stimulus trains yielded similar responses in slices from 3-month-old small t-expressing and controls animals (Figure 4G; Table S2; 189% ± 8% of baseline at 100–120 min for double transgenics versus 187% ± 10% for controls, $p = 0.86$, $5/5$, $5/5$), and we also observed similar responses to a single 1 s, 100 Hz stimulus train (Figure 4H; Table S2; 123% ± 4% of baseline at 100–120 min for double transgenics versus 110% ± 2% for controls, $p = 0.07$, $n = 8/5$, $8/5$). That LTP in response to a number of different stimulus protocols is apparently unaffected by small t expression is in stark contrast to the blockade of both stimulus and chemically induced LTD we observe, suggesting that the LTD deficit in these animals is not accompanied by a metaplastic shift.

Small t Expression Does Not Affect Depotentiation

Depotentiation is a decrease in synaptic efficacy that reverses the effects of potentiation. Like LTD, depotentiation can be

elicited by low frequency stimulus trains and requires NMDAR activation and phosphatase activity (Fujii et al., 1991; O'Dell and Kandel, 1994). However, these two processes also differ in important ways. Depotentiation is dependent on prior potentiating stimuli for expression, whereas LTD can be elicited at naive synapses (Barrionuevo et al., 1980; Fujii et al., 1991; O'Dell and Kandel, 1994; Staubli and Lynch, 1990). Furthermore, the identification of substrates that are differentially modified during depotentiation and LTD and manipulations that selectively inhibit depotentiation show that there are also differences at the molecular level, in the pathways that mediate the two processes (Lee et al., 2000; Zhuo et al., 1999). We therefore sought to determine if small t expression affected depotentiation in addition to LTD.

As with other forms of LTP, three 1 s trains of 100 Hz given at 20 s intervals elicited potentiation at Schaffer collateral synapses in both small t-expressing and control slices (Figure 5A; 167% ± 3% of baseline at 40–60 min after stimulation for double transgenics versus 162% ± 3% for controls, $p = 0.11$, $n = 10/5$, $10/5$). These responses returned to baseline when a single 1 Hz stimulus train was applied to this same pathway (Figure 5B; 99% ± 1% of baseline at 25–35 min for double transgenics versus 98% ± 1% for controls, $p = 0.99$, $n = 15/8$, $14/8$), suggesting that unlike LTD, depotentiation was not affected by small t expression (see also Figures S2C–S2E).

Previous observations have demonstrated a time dependence for depotentiation, such that the depotentiating low-frequency stimulus train must be delivered within a critical time period following potentiation (Fujii et al., 1991; O'Dell and Kandel, 1994). When we delivered a second low-frequency stimulus train to the depotentiated pathway 35 min after potentiation, we observed a further decrease in evoked responses in slices from control animals that was blocked by small t expression (Figure 5B; 98% ± 1% of baseline at 60–70 min for double transgenics versus 76% ± 1% for controls, $p = 0.0001$). The failure of the second LFS train to elicit a further decrease in evoked responses in small t-expressing slices is consistent with our earlier demonstration of an LTD deficit in these animals and shows that depotentiation is intact in the same pathway, in the same slices, and under the same conditions where we observe this LTD impairment.

Small t Expression Does Not Affect mGluR-Dependent LTD

Two different forms of LTD coexist at Schaffer collateral/CA3 synapses, one dependent on NMDA receptors and another dependent on mGluRs. These two forms can be experimentally dissociated from one another by altering the $Ca^{2+}:Mg^{2+}$ ratio in the solution bathing the slices during recording (Oliet et al., 1997; Figures S2F–S2I; Experimental Procedures). Our earlier experiments evoked a form of LTD that was dependent on NMDAR activity. We therefore utilized this modified protocol to test the effects of small t on the mGluR-dependent form of LTD. Consistent with previous reports showing that mGluR-dependent LTD at this synapse does not depend on serine/threonine phosphatase activity (Schnabel et al., 2001), we found that 1 Hz, 15 min stimulus trains elicited a persistent depression in both small t-expressing and control slices (Figure 5C; 58% ± 2% of baseline at 100–120 min for double transgenics versus 56% ± 9% for controls, $p = 0.90$, $n = 6/6$, $6/6$). The

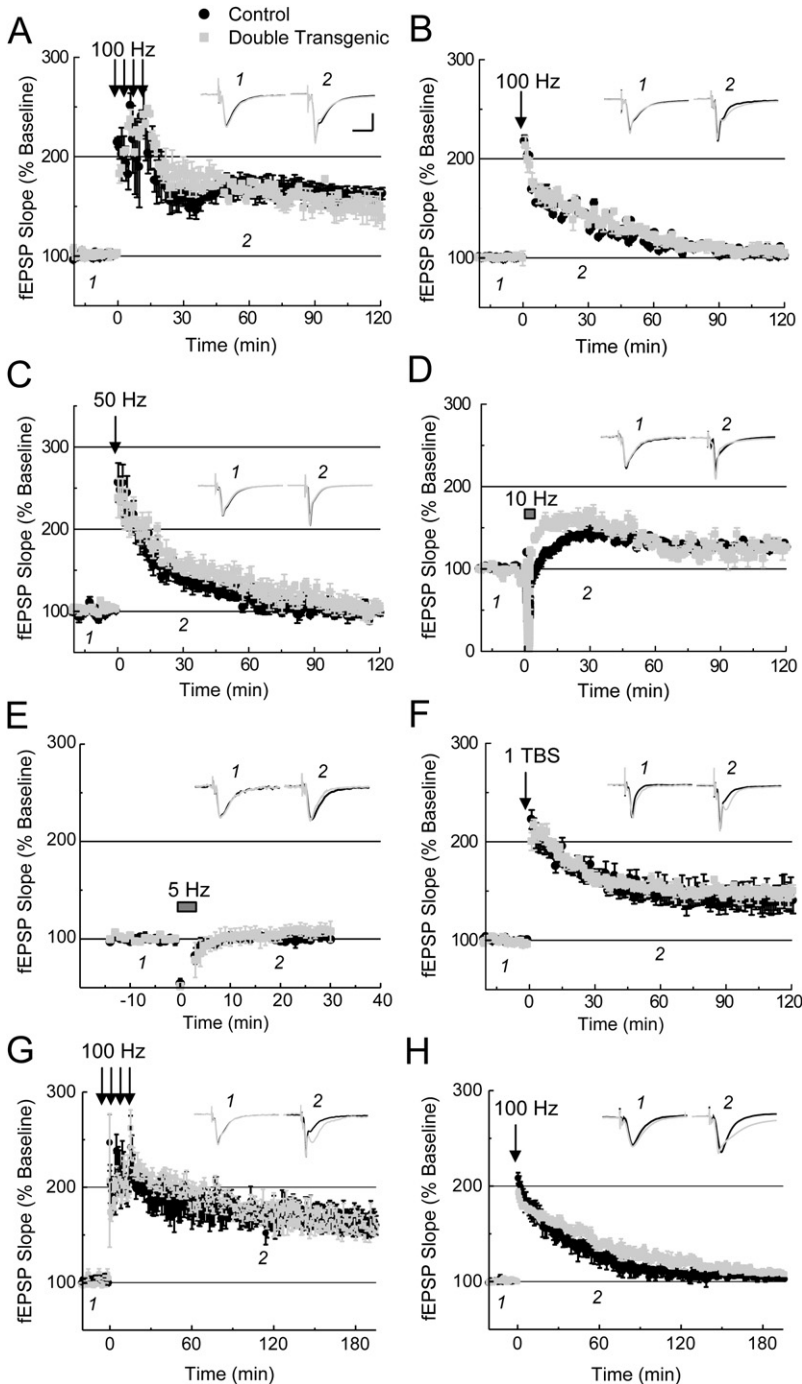


Figure 4. Potentiation Is Normal in Small t-Expressing Mice

Averaged initial fEPSP slopes \pm SEM obtained at hippocampal Schaffer collateral/CA1 synapses in slices from 21- to 28-day-old animals (A–F) or 3-month-old adults (G and H) in response to the following stimulation protocols: (A) 4 × 1 s, 100 Hz trains, (B) 1 × 1 s, 100 Hz train, (C) 1 × 1 s, 50 Hz train, (D) 1 × 90 s, 10 Hz train, (E) 1 × 3 min, 5 Hz train, (F) 9 bursts of 100 Hz given at 5 Hz, (G) 4 × 1 s, 100 Hz trains, (H) 1 × 1 s, 100 Hz train. Insets show sample traces taken before (1) and after stimulation (2) for double-transgenic animals (gray) and controls (black).

small t expression blocks the NMDAR-dependent form of LTD it does not affect the mGluR dependent form.

Small t Expression Blocks NMDA-Induced But Not DHPG-Induced AMPA Receptor Internalization

One of the mechanisms whereby LTD is expressed is via the regulated internalization of AMPA receptors (Carroll et al., 1999; Snyder et al., 2001). To determine if the specific NMDAR-dependent deficit, we observed at the physiological level was also reflected at the cellular level in the form of impaired receptor internalization, we labeled surface-expressed AMPA receptors in dissociated cultures of hippocampal neurons transduced with either small t-expressing or control viral vectors and applied 20 μ M NMDA for 1 min. This treatment resulted in a 52% reduction in surface labeled GluR2-containing AMPA receptors in cells transduced with control vector, while this same treatment resulted in no internalization in cells expressing small t (Figures 6A and 6B; control viral vector, 48.17% \pm 6.79% compared to vehicle treated control cells; small t, 104.10% \pm 7.48%). In contrast, a 10 min application of the group 1 mGluR agonist, DHPG (20 μ M), resulted in comparable AMPAR internalization in both small t-expressing and control cells (Figures 6A and 6B; control viral vector, 47.87% \pm 6.49% compared to untreated control cells; small t, 34.86% \pm 5.79%). This deficit in NMDA-induced but not DHPG-induced AMPA receptor internalization

mGluR-dependent form of LTD can also be elicited by bath application of the group I mGluR agonist, DHPG (Palmer et al., 1997). We similarly found that a 20 min application of 100 μ M DHPG to acute slices prepared from adult animals resulted in a persistent depression of Schaffer collateral evoked responses that was comparable in small t-expressing and control slices (Figure 5D; 55% \pm 4% of baseline at 100–120 min post-drug application for double transgenics versus 63% \pm 3% for controls, $p = 0.27$, $n = 7/5$, 7/5). These results suggest that while

perfectly mirrored the deficit in NMDAR-dependent but not mGluR-dependent LTD that we observe on the physiological level lending additional support to the notion that small t expression selectively blocks NMDAR-dependent LTD.

Small t-Expressing Animals Exhibit a Reversal Deficit in the Morris Water Maze

Small t expression results in an absence of both stimulus and chemically induced LTD at Schaffer collateral synapses that is

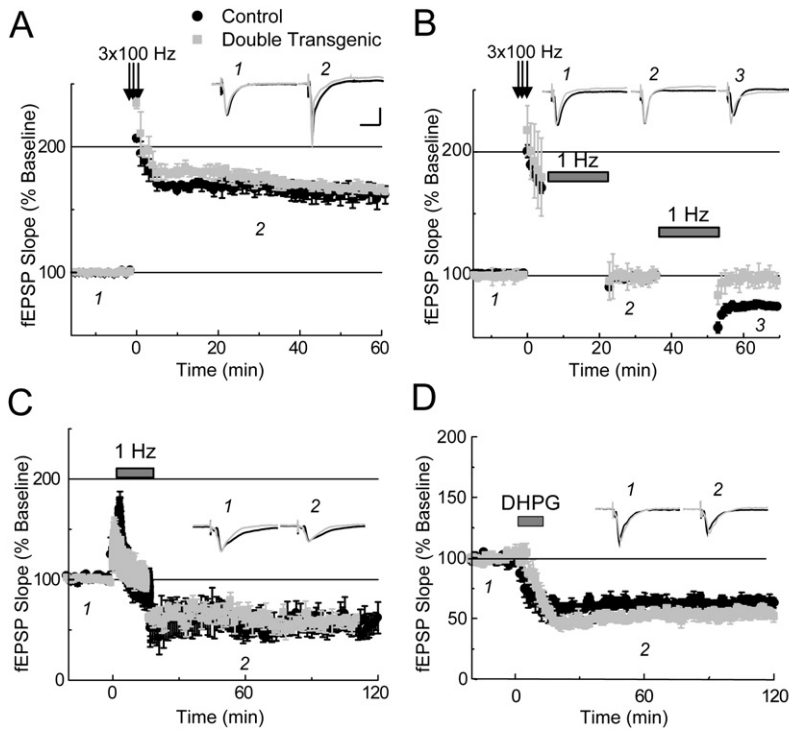


Figure 5. Depotentiation and mGluR-Dependent LTD Are Normal in Small *t*-Expressing Mice

Average potentiated responses \pm SEM elicited following 3×1 s, 100 Hz trains in slices from 21- to 28-day-old animals (A) is reversed in control and double-transgenic slices by a single 15 min, 1 Hz train delivered 5 min following potentiation (B), while a second 15 min, 1 Hz train delivered 35 min following potentiation elicits LTD in control but not double-transgenic slices.

(C) Average responses \pm SEM for mGluR-dependent, NMDAR-independent LTD induced by a 15 min, 1 Hz stimulus train in modified ACSF in slices from 21- to 28-day-old animals.

(D) Average responses \pm SEM for mGluR-dependent LTD induced by a 20 min application of 100 μ M DHPG in slices from 3-month-old animals. Insets show sample traces taken at the times indicated for double-transgenic animals (gray) and controls (black).

not accompanied by changes in potentiation, depotentiation, or mGluR-dependent LTD. To determine what, if any, behavioral phenotype might be associated with this highly selective physiological deficit, we first compared the behavior of small *t*-expressing and control animals in a visible version of the Morris water maze. We found that both groups learned to swim to the marked platform equally well, with the average path lengths decreasing from 704 ± 76 cm and 890 ± 96 cm on the first day to 395 ± 51 cm and 334 ± 42 cm on the second day for double transgenics and controls, respectively (Figure 7A; days 1–2). As with the hidden version of this task below, these similarities in path lengths were accompanied by similar swimming speeds and latencies (data not shown).

We next trained these animals to swim to a hidden platform located in a fixed location of the pool (Figure 7A, days 3–12). Again

$p < 0.0001$, but not genotype, $p = 0.25$) as well as the time spent in the platform-containing quadrant during probe trials on days 7 ($34\% \pm 4\%$ of time in training quadrant for double transgenics versus $36\% \pm 5\%$ for controls; $p = 0.78$) and 12 (Figure 7C, $44\% \pm 4\%$ of time in training quadrant, TQ, for double transgenics; $46\% \pm 5\%$ for controls, $p = 0.83$).

We then moved the hidden platform to the opposite quadrant of the pool and trained the animals to swim to this new location (Figure 7A; days 13–17). Both the small *t*-expressing and control animals learned to swim to the new platform location reaching similar path lengths by the final day of training, day 17 (Figure 7A; 263 ± 89 cm for double transgenics versus 243 ± 28 for controls, $p = 0.20$), and exhibiting similar preferences for the new training quadrant during a probe trial given on day 17 (Figure 7D; $35\% \pm 2\%$ of time in new training quadrant, TQ, for

animals expressing small *t* acquired this task at the same rate as controls and performed equally well once the task was acquired. This was apparent in the similar decrease over time in the average distance the animals traveled to locate the platform (repeated-measures ANOVA days 3–12 revealed significant effect of training day,

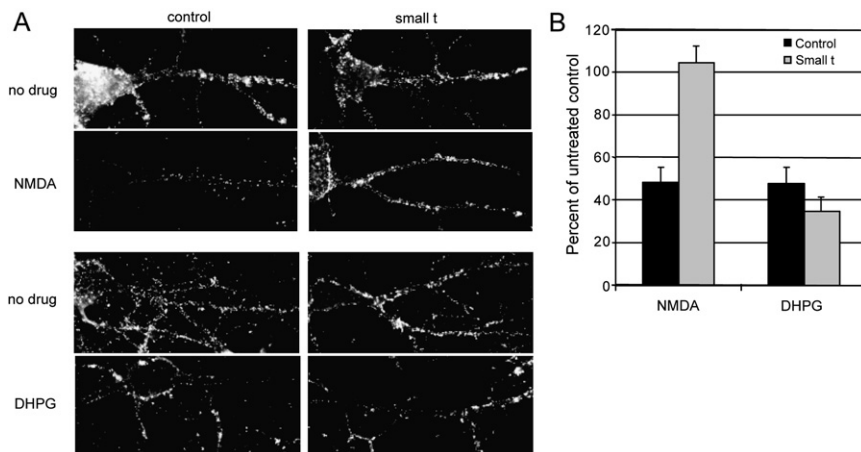


Figure 6. Small *t* Expression Selectively Blocks NMDA-Induced AMPA Receptor Internalization

(A) Representative images showing surface labeled GluR2-containing AMPARs in cells transfected with small *t*-expressing or control vector following treatment with NMDA (upper four panels) or DHPG (lower four panels).

(B) Averaged data \pm SEM for seven NMDA-induced and four DHPG-induced internalization experiments expressed as percent of surface labeling in untreated controls.

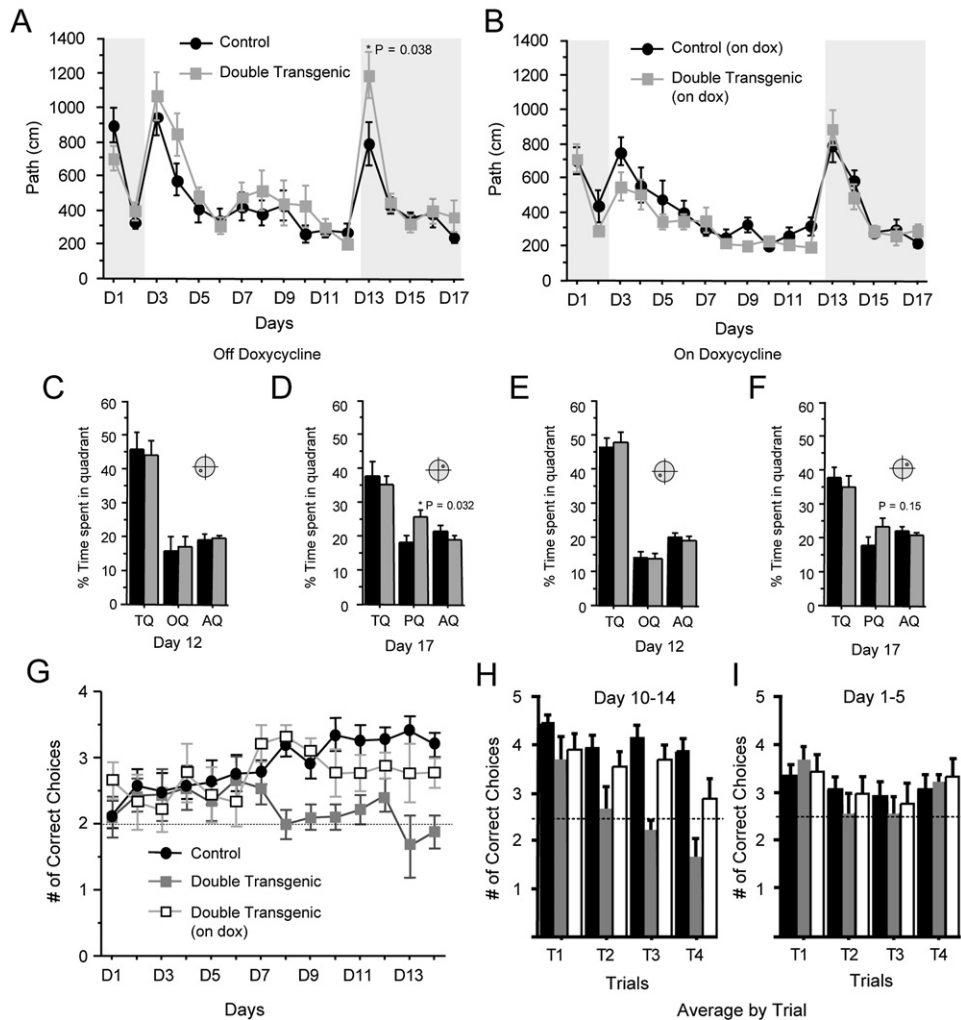


Figure 7. Small t-Expressing Mice Exhibit Deficits in Behavioral Flexibility

(A) Average distance traveled to the platform \pm SEM in the Morris water maze during the visible platform (days 1 and 2), hidden platform (days 3–12), and reversal (days 13–17) phases of the task ($n = 15$ for double transgenic and 16 for controls).

(B) Plot as described in (A) but for animals shifted to doxycycline-containing food at 6 weeks of age ($n = 15, 15$).

(C) Averages \pm SEM for percent of time spent in the initial training quadrant (TQ), the opposite quadrant (OQ), and the average of the two adjacent quadrants (AQ) during probe trials given on day 12 of experiment shown in (A).

(D) Averages \pm SEM for percent of time spent in the new training quadrant (TQ), the previous training quadrant (PQ), and the average of the two adjacent quadrants (AQ) during probe trials given on day 17 of experiment shown in (A).

(E–F) Correspond to data shown in (C) and (D), respectively, but for animals described in (B). In (C)–(F), gray bars represent average for double transgenics and black bars controls.

(G) Average number of correct choices \pm SEM per day in a delayed nonmatch to place T-maze task for double transgenic (gray squares, $n = 9$) and control animals (filled circles, $n = 14$), and double-transgenic animals shifted to doxycycline-containing food at 6 weeks of age (open squares, $n = 9$).

(H and I) Average number of correct choices made by animals described in (G) on trials 1, 2, 3, and 4 (T1, T2, T3, and T4) of days 10–14 (H) and days 1–5 (I).

double transgenics; $38\% \pm 4\%$ for controls, $p = 0.63$). However, small t antigen-expressing animals acquired this new location more slowly (repeated-measures ANOVA days 13–17 revealed significant effects of both training day, $p < 0.0001$ and genotype, $p = 0.037$) exhibiting significantly longer path lengths on the first day of training in the new platform location (Figure 7A; 1187 ± 134 cm for double transgenics on day 13 versus 787 ± 125 cm for controls, $p = 0.038$) that were not apparent on the first day of training for the original platform location (Figure 7A;

1066 ± 142 cm for double transgenics on day 3 versus 944 ± 104 cm for controls, $p = 0.49$). In addition, this delay in learning the new platform location was accompanied by an increase in the percent of time the animals spent searching for the platform in the previously correct quadrant during a probe trial given on day 17 (Figure 7D; $26\% \pm 2\%$ of time in previously correct quadrant, PQ, for double transgenics versus $18\% \pm 2\%$ for controls, $p = 0.032$). This increased perseverance together with the increased path lengths on the first day of reversal suggest that

while small *t* expression does not appear to affect acquisition of the hidden platform water maze task, it does appear to result in impaired behavioral flexibility when the location of the platform is changed during the reversal phase of this task.

Above, we showed that the adult deficits in stimulus-induced and chemically induced LTD depend on the adult expression of small *t* antigen. To determine if this was also the case for the reversal deficit in the Morris water maze, we repeated the same visible platform, hidden platform, and reversal experiment on double-transgenic and control animals that were shifted onto doxycycline-containing food at 6 weeks of age. As before, we found that both groups of animals acquired the hidden platform task equally well (Figure 7B; repeated-measures ANOVA days 3–12 revealed significant effect of training day, $p < 0.0001$ but not genotype, $p = 0.25$) and showed similar preferences for the training quadrant during probe trials given on days 7 ($42\% \pm 4\%$ of time in training quadrant for double transgenics versus $42\% \pm 4\%$ for controls, $p = 0.98$) and 12 (Figure 7E; $48\% \pm 3\%$ of time in training quadrant, TQ, for double transgenics versus $46\% \pm 3\%$ for controls, $p = 0.66$). However, suppressing adult expression of small *t* by doxycycline administration eliminated the previously observed differences in learning a new platform location (repeated-measures ANOVA for days 13–17 revealed significant effect of training day, $p < 0.0001$ but not genotype, $p = 0.90$), in path length on the first day of reversal (Figure 7B; 887 ± 103 cm on day 13 for double transgenics versus 790 ± 99 cm for controls, $p = 0.51$) and in perseverance in the previously correct quadrant during a day 17 probe trial (Figure 7F; day 17: $23\% \pm 3\%$ of time in previously correct quadrant, PQ, for double transgenics versus $18\% \pm 2\%$ for controls, $p = 0.15$). These results suggest that like the physiology deficit, the reversal deficit in the water maze, also depends on the contemporaneous expression of small *t*.

Small *t*-Expressing Animals Exhibit a Deficit in a Delayed Nonmatch to Place T-Maze Task Due to Increased Intertrial Interference

The delayed nonmatch to place T-maze task is another hippocampus-dependent spatial task commonly used in rodents (Bannerman et al., 1999, 2004; Hock and Bunsey, 1998; McHugh et al., 2008). Like the reversal phase of the water maze above, normal performance in this task also requires behavioral flexibility since the animals must learn and remember new spatial information as the locations of rewards change between trials. We therefore compared the ability of small *t*-expressing and control animals to learn a delayed nonmatch to place T-maze task. While control animals gradually learned this task, reaching a plateau in performance of 3.31 ± 0.11 average correct choices over the last 5 days of the experiment (Figure 7G, days 10–14, filled circles), small *t*-expressing animals continued to perform at chance over the same time period (Figure 7G, gray squares; 2.07 ± 0.63 average correct choices, days 10–14, $p < 0.001$ Scheffe's post-hoc analysis for double transgenic versus control). Here again the deficit in the delayed nonmatch to place T-maze task was dependent on the adult expression of small *t*, since it could be suppressed by shifting animals onto doxycycline-containing food beginning at 6 weeks of age (Figure 7G, open squares; 2.80 ± 0.24 average correct choices on days 10–14, $p = 0.026$

Scheffe's post-hoc analysis for double transgenic shifted onto dox versus off dox, $p = 0.16$ for double transgenic shifted onto dox versus control).

To more directly assess the ability of the small *t*-expressing animals to learn new spatial information, we analyzed their performance by trial rather than by day over the last 5 days of the experiment (days 10–14). We found that while the small *t*-expressing animals performed normally on the first trial of a day, when the most recent trial was 24 hr earlier, their performance dropped to chance levels during trials 2, 3, and 4, when the most recent trial was only 15 min earlier (Figure 7H, gray bars; Scheffe's post-hoc analysis for day 10–14 double transgenics versus controls on trial 1, $p = 0.28$; trial 2, $p = 0.04$; trial 3, $p < 0.001$; trial 4, $p < 0.001$). This pattern is consistent with the phenomenon of intertrial interference and suggests that information learned by these animals during recently experienced trials impaired their performance on subsequent trials (Aultman and Moghaddam, 2001). This intertrial interference was reduced when the double transgenic animals were shifted to doxycycline-containing food at 6 weeks of age, suggesting that it too is dependent on contemporaneous expression of small *t* (Figure 7H, days 10–14, white bars). This pattern was not observed in the small *t* antigen-expressing animals during the early stages of the experiment, before they acquired the task (Figure 7I, days 1–5), and it was not observed over the 4 trials of a day in a version of the task where the location of the food reward was fixed (Figures S4G and S4H), suggesting that it is not due to a decrease in motivation across trials. Finally, the delayed nonmatch to place T-maze task deficit we observe in animals that express small *t* antigen was not associated with any differences in their behavior in an open field such as locomotion or exploratory activity (Figures S4A–S4F).

Small *t*-Expressing Animals Exhibit Normal Retention of Spatial Memory in the Morris Water Maze

One explanation for the behavioral flexibility deficits we observe in the small *t*-expressing animals in the water maze and T maze is that they reflect enhanced memory. If an animal learns something too well initially, then it may be harder to override that memory when the location of a reward (platform or food) changes. To explore this possibility, we looked for evidence of memory enhancement by training a group of animals in an abbreviated version of the Morris water maze hidden platform task. As was the case for the experiment described in Figure 7A, the distance traveled to a hidden platform decreased over the course of 4 days for both small *t*-expressing and control animals (Figure 8A; 912 ± 110 cm and 785 ± 88 cm on day 3 to 405 ± 49 cm and 514 ± 86 cm on day 6 for double transgenics and controls, respectively; repeated-measures ANOVA revealed a significant effect of training day, $p < 0.0001$, but not genotype, $p = 0.76$). Following these 4 days of training, the animals were returned to their home cages until they were tested again in probe trials given 2 and 4 weeks later. Two weeks after training, small *t*-expressing and control animals showed similar preferences for the training quadrant, suggesting that both groups retained the location of the escape platform equally well (Figure 8B; $39\% \pm 3\%$ of time spent in training quadrant, TQ, for double transgenics versus $39\% \pm 2\%$ for controls, $p = 0.98$). When

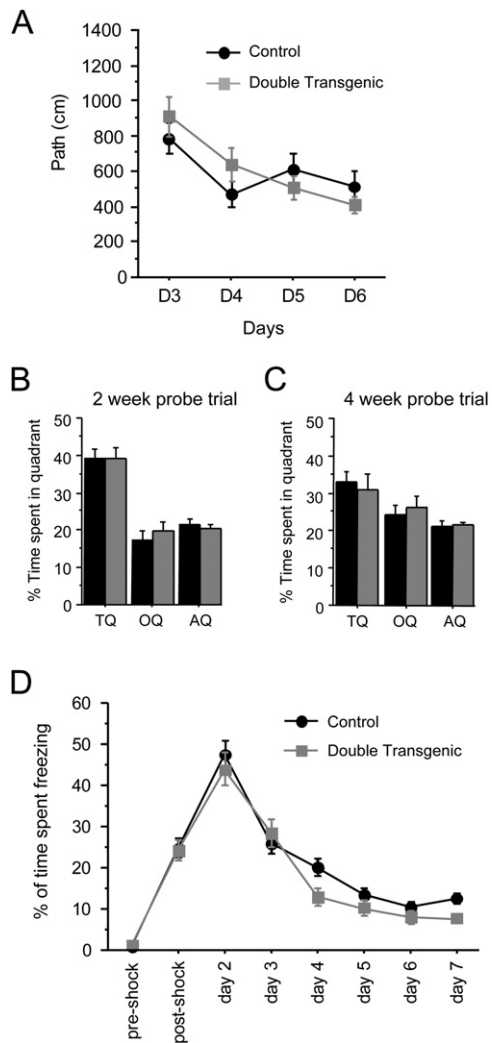


Figure 8. Normal Spatial Memory Retention and Extinction of Contextual Fear Conditioning in Small *t*-Expressing Mice

(A) Average distance traveled to the hidden platform \pm SEM in the Morris water maze as function of training day for double transgenic and control animals ($n = 15, 16$).

(B and C) Averages \pm SEM for percent of time double transgenic (gray bars) or control animals (black bars) spent in the training quadrant (TQ), the opposite quadrant (OQ), and the average of the two adjacent quadrants (AQ) of the water maze during probe trials given 2 (B) and 4 (C) weeks after the training shown in (A).

(D) Plot of the average percent of time \pm SEM double transgenic (gray squares, $n = 13$) and control animals (filled circles, $n = 15$) spent freezing during the indicated stages of a contextual fear conditioning/extinction protocol.

both groups were again tested in probe trials given 4 weeks after training, their preference for the training quadrant decreased to $31\% \pm 4\%$ for animals expressing small *t* and $33\% \pm 3\%$ for controls (Figure 8C), suggesting that their memories for the platform location had degraded over time. However, the fact that this decrease occurred to a similar extent in both groups ($p = 0.64$) suggests that small *t* expression afforded no improvement in the memory of the platform location in this task.

Small *t*-Expressing Animals Exhibit Normal Extinction of Contextual Fear Conditioning

Contextual fear conditioning is a form of classical conditioning in which an animal learns to associate an aversive stimulus (in this case a mild electric shock) with a particular environment. Subsequent exposure of the animal to the environment in the absence of the shock then elicits a fear response (measured by freezing) that decreases over time. This decrease is referred to as extinction and this process is a type of behavioral flexibility that has been studied extensively (Ji and Maren, 2007). We sought to determine if small *t* expression also affected flexibility in this task. To do this, we exposed animals to a novel environment and measured the percent of time spent freezing before and after 3×2 s foot shocks. Small *t*-expressing and control animals showed equivalent levels of freezing both before and after the shocks, as well as during re-exposure to the same environment in the absence of shock 24 hr later (Figure 8E; $1.3\% \pm 0.4\%$ versus $0.9\% \pm 0.2\%$ preshock, $p = 0.37$; $24.2\% \pm 2.5\%$ versus $24.7\% \pm 2.4\%$ postshock, $p = 0.89$; and $43.9\% \pm 3.9\%$ versus $47.4\% \pm 3.6\%$ on day 2, $p = 0.52$; for double transgenics and controls, respectively). Repeated re-exposure once every 24 hr for 5 days resulted in a decrease in freezing that was comparable in small *t*-expressing and control animals, suggesting that small *t* expression does not affect this form of behavioral flexibility (repeated-measures ANOVA for effect of genotype, $p = 0.17$).

DISCUSSION

LTD and Behavior

LTD plays a central role in the cerebellum in several forms of motor learning (Boyden et al., 2004). In the cerebral cortex, LTD is involved in the formation of ocular-dominance columns (Heynen et al., 2003; Rittenhouse et al., 1999) and whisker-receptive fields (Allen et al., 2003; Feldman and Brecht, 2005). In the perirhinal cortex, LTD is thought to be required for the appropriate response of cells in this structure to novel visual stimuli (Warburton et al., 2003). In the nucleus accumbens, LTD has been implicated in addiction (Brebner et al., 2005; Kourich et al., 2007; Thomas et al., 2001). In the hippocampus, studies have suggested that a metaplastic shift toward LTD may be important for novelty detection (Kemp and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 1999) or for habituation (Etkin et al., 2006). In this study, we used genetic methods to selectively block the NMDAR-dependent form of LTD and examine its behavioral role in spatial learning and memory.

We found that expression of a PP2A-inhibitory fragment of SV40 small *t* antigen produced a highly selective and reversible blockade of NMDA receptor-dependent LTD at Schaffer collateral synapses that was not accompanied by alterations in potentiation, depotentiation, or mGluR-dependent LTD. This physiological deficit was also reflected on the cellular level by a blockade of NMDA-induced but not DHPG-induced AMPAR internalization. The specific loss of NMDA receptor-dependent LTD in these animals was accompanied by a deficit in behavioral flexibility that was evident in two different tasks. In the Morris water maze, these animals learn normally but exhibit both delayed acquisition of a new platform location and perseveration in the previous location during the reversal phase of this experiment.

In the delayed nonmatch to place T-maze task, these animals also exhibit a flexibility deficit in the form of enhanced intertrial interference, performing normally on the first trial of a day but exhibiting impaired performance on subsequent trials as the location of the food reward is changed. The correlation between a highly specific physiological deficit in these animals and these behavioral phenotypes suggests that NMDAR-dependent LTD is required for normal behavioral flexibility in these tasks.

The Link between the Water Maze and T Maze Phenotypes

Intertrial interference in the delayed nonmatch to place T-maze task can also be produced in wild-type mice by shortening the intertrial interval (Aultman and Moghaddam, 2001). Like the small *t*-expressing mice, wild-type animals subjected to this shortened interval exhibit normal performance on the first trial of a day and impaired performance on subsequent trials. This distinct pattern of behavior is thought to reflect the influence of previously learned information from earlier trials on performance in subsequent trials. Since spatial memory in this task is a short-term form of memory, the interference is thought not to occur at normal intertrial intervals or on the first trial of a day (when the effective intertrial interval is ~24 hr) because the interfering information has been forgotten during that time.

Several aspects of this intertrial interference phenotype, therefore, are reminiscent of the reversal phenotype that the small *t*-expressing animals exhibit in the water maze. In the water maze, small *t*-expressing animals acquire an initial platform location normally, and this may be analogous to the first trial of a day in the T-maze since in both cases the animals are effectively naive about the location of the reward (food or platform). In contrast to initial acquisition in the water maze, small *t*-expressing animals exhibit a deficit during reversal when the platform location is moved, and this may be analogous to the trials 2–4 of a day in the T maze since in both cases the animals show deficits only when previous information has been learned and the location of the reward is changed. Finally, the perseverance phenotype we observe in the small *t*-expressing animals during the day 17 probe trial also parallels the intertrial interference phenotype we observe in the T maze since it suggests that information pertaining to the previously learned platform location may contribute to the reversal deficit.

Both the T maze and water maze tasks require hippocampal function; however, since our current genetic manipulation is only restricted to principal cells in the forebrain it is possible that the similar behavioral phenotypes in these two tasks arise from deficits in distinct structures. It will therefore be interesting to further restrict the expression of small *t* within the forebrain in order to associate the behavioral consequences of NMDA receptor-dependent LTD blockade with its actions at specific synapses or sets of synapses. This approach may also prove useful in investigating the role of LTD in different structures and behaviors as outlined above and may also facilitate a direct physiological visualization of LTD in behavioral flexibility.

The Link between LTD and Behavioral Flexibility

If the deficit in flexibility we observe in the small *t*-expressing animals is in fact due to a deficit in NMDAR-dependent LTD or

its *in vivo* correlate, then similar behavioral deficits should be observed in other animals carrying manipulations that disrupt this same form of plasticity. One example where this may be the case is afforded by mice carrying a knockout mutation of one of the calcineurin regulatory subunits that is restricted to principal cells in the forebrain (Zeng et al., 2001). While originally interpreted as a working memory-like deficit, these mice exhibit a behavioral phenotype that is strikingly similar to the phenotype we describe for animals that express small *t* in these same cells. This phenotype includes a deficit in reversal in the Morris water maze and a deficit in the radial arm maze that may be related to the T-maze deficit we observe. Interestingly, this behavioral phenotype occurs in conjunction with a selective LTD deficit. While this deficit is not as complete as the one we observe in the small *t*-expressing animals, and the physiological consequences of the knockout include modest enhancements in LTP, the similarities between the phenotypes in these two animals suggests that a deficit in LTD may underlie the flexibility deficits in both cases.

This association between LTD and behavioral flexibility has also received recent support from the work of Duffy et al. (2007) that links *enhanced* LTD with an *improvement* in spatial reversal learning. These authors found that bath application of the NMDA receptor coagonist, D-serine, to acute hippocampal slices from wild-type mice had the opposite effect of small *t* expression, selectively enhancing LTD without affecting potentiation. They then went on to show that systemic administration of D-serine enhanced performance during the reversal phase of the Morris water maze task but not during the initial acquisition of this task; results which also mirror the consequences of small *t* expression on the behavioral level. Duffy et al. (2007) further report that systemic administration of Ro 25-6891, an inhibitor of NR2B-containing NMDA receptors, caused an impairment in reversal learning in the water maze. Initially, the controversy surrounding the physiological effects of this inhibitor, which has been variously reported to selectively inhibit LTD (Liu et al., 2004), have no effect on LTD (Morishita et al., 2007), both enhance LTD and inhibit LTP (Fox et al., 2006), and selectively inhibit LTP (Bartlett et al., 2007), has made it difficult to correlate its behavioral actions with an effect on a particular type of synaptic plasticity. However, the similarity between the behavioral consequences of Ro 25-6891 administration and the behavioral phenotype present in the small *t* antigen-expressing mice suggests that it too may affect behavioral flexibility by inhibiting LTD.

How Might NMDAR-Dependent LTD Be Involved in Flexibility?

One possible explanation for the flexibility deficit we observe in the small *t*-expressing mice is that it reflects a role for LTD in normal learning that is apparent only when memory demands are high, as may be the case for reversal in the water maze or the delayed nonmatch to place T-maze task. This could be due to the proposed function of LTD in increasing the signal-to-noise ratio between potentiated synapses that participate in a memory trace and those that do not. This idea is supported by physiological observations made in animals exposed to novel environments where exposure to these environments facilitated stimulus-induced LTD and reversed previously elicited potentiation

(Kemp and Manahan-Vaughan, 2004; Lemon and Manahan-Vaughan, 2006; Manahan-Vaughan and Braunewell, 1999; Xu et al., 1998). The reported increase in memory retention that results from pharmacological inhibition of NMDA-dependent forms of plasticity (including LTD) may also reflect the ability of LTD to reverse potentiation at synapses that participate in spatial memory (Villarréal et al., 2002).

In the models outlined above, LTD could perform this function by acting broadly at all nonpotentiated synapses in a structure. However, the form of NMDAR-dependent LTD that we describe here has been found to act homosynaptically at synapses subjected to particular patterns of stimulation (Mulkey and Malenka, 1992). It is therefore tempting to think that LTD may be acting at particular synapses to enhance the signal-to-noise ratio of a memory trace. If this occurs selectively at synapses responsible for encoding information that becomes irrelevant with new learning (such as the previous locations of escape platforms or food rewards), then this specificity could explain how information about a location or task could be modified without affecting unrelated memories. According to this theory, LTD may function to weaken previous memory traces, thereby preventing those traces from interfering with newly encoded information when the demands of a task change. This might also explain the lack of an effect of the LTD deficit on extinction, since evidence suggests that extinction occurs via a mechanism in which the underlying memory trace remains largely intact (Ji and Maren, 2007).

EXPERIMENTAL PROCEDURES

Generation of Transgenic Mice and Analysis of Transgene Expression

A fragment encoding the C-terminal 86 amino acids of SV40 small t antigen was amplified from the plasmid pB-stA (provided by Marc Mumby) by PCR, fused at the N terminus to an HA-epitope tag, and used to generate an adenoviral vector as described previously (He et al., 1998). Immunoprecipitation experiments were then carried out on HeLa cells transduced with this vector using an anti-PP2A/A subunit antibody followed by western blot analysis with this antibody as well as antibodies against small t antigen and the PP2A/C subunit. This same coding sequence was then inserted into the tetO vector, pMM400, (Mayford et al., 1996) for generation of transgenic mice as described previously (Hogan et al., 1994). Distribution of small t antigen RNA in fresh frozen sections was analyzed by *in situ* hybridization as described previously (Wisden and Morris, 2002). Protein expression was assessed by western blots of hippocampal homogenates using anti-small t (Oncogene Research, Boston, MA) and anti- β -tubulin (Sigma, St. Louis, MO) antibodies. See also supplemental methods.

Electrophysiology

Field EPSP recordings were conducted on transverse hippocampal slices (400 μ m) incubated in an interface chamber as described previously (Barco et al., 2002) and in Supplemental Experimental Procedures. Fostriecin was from Sigma (St. Louis, MO), NMDA from Calbiochem (La Jolla, CA), DHPG, MCPG, and D-AP5 from Tocris (Ellisville, MO). Modified ACSF for stimulus-induced mGluR-dependent LTD experiments was prepared by reducing the CaCl_2 concentration in the standard recipe to 1.25 mM. For electrophysiological experiments, *n* indicates the number of slices followed by the number of animals.

AMPA Internalization Assay in Hippocampal Cultures

HSV amplicons expressing the HA epitope-tagged C-terminal 86 amino acids of SV40 small t antigen were generated in the vector pHGCX (Saeki et al., 2001) and prepared as described previously (Bowers et al., 2001). Lentiviral vectors generated in the vector MA1 (Amendola et al., 2005) were prepared using the

Virapower Expression system (Invitrogen, Carlsbad, CA). Both vectors were used to transduce hippocampal cultures prepared from P0 rat pups. Experiments performed using HSV and lentiviral vectors gave indistinguishable results, which were pooled. Cultures were incubated in media with an anti-GluR2 antibody (Chemicon, Temulca, CA), recognizing an N-terminal epitope. Receptor internalization was induced by 1 min treatment with 20 μ M NMDA plus 10 μ M CNQX, 1 μ M MPEP, and 1 μ M LY367385 (Tocris Cookson, Inc., Ellisville, MO) or 10 min with 20 μ M DHPG plus 10 μ M CNQX and 50 μ M APV (Tocris) at 37°C. *n* values represent individual experiments in which 7–15 cells were imaged in each condition. See also Supplemental Experimental Procedures.

Behavior

For all behavioral tasks, 3-month-old male double-transgenic and single-transgenic control littermates backcrossed to C57BL6J for at least five generations were used. ANOVAs were conducted on the behavioral data with genotype as the between-subject factor, and data are presented as mean \pm SEM. The experimenter was blind to the genotype in all studies.

Water-Maze Task

The water-maze task was performed as previously described (Malleret et al., 2001). Four trials were given each day with a 15 min intertrial interval. Probe trials in which the escape platform was removed from the pool were conducted on days 7, 12, and 17. A second water-maze task was carried out on a separate group of animals essentially as described above, except that no transfer phase was carried out, and the acquisition phase consisted of only 4 days of training followed by probe trials given 2 and 4 weeks later.

Delayed Nonmatch to Place T-Maze Task

This task was performed as previously described (Kellendonk et al., 2006) with 4 trials per day, delays of approximately 5 s, and intertrial intervals of 15 min.

Contextual Fear Conditioning and Extinction

Experiments were done as described previously (Shumyatsky et al., 2005). All trials consisted of a 6 min exposure to a conditioning chamber once per day for 7 days. The first trial consisted of a 2 min of exposure (preshock) followed by 3 tone/shock pairings (30 s of tone at 2800 Hz, 85 dB, the last 2 s of which were paired with a 2 s 0.7 mA continuous foot shock). On subsequent days, animals were reintroduced to the conditioning chamber in the absence of tone or shock. See also Supplemental Experimental Procedures.

SUPPLEMENTAL DATA

The Supplemental Data for this article can be found online at <http://www.neuron.org/cgi/content/full/58/1/104/DC1/>.

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