Development/Plasticity/Repair

Endogenous Truncated TrkB.T1 Receptor Regulates Neuronal Complexity and TrkB Kinase Receptor Function *In Vivo*

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Pathological or *in vitro* overexpression of the truncated TrkB (TrkB.T1) receptor inhibits signaling through the full-length TrkB (TrkB.FL) tyrosine kinase receptor. However, to date, the role of endogenous TrkB.T1 is still unknown. By studying mice lacking the truncated TrkB.T1 isoform but retaining normal spatiotemporal expression of TrkB.FL, we have analyzed TrkB.T1-specific physiological functions and its effect on endogenous TrkB kinase signaling *in vivo*. We found that TrkB.T1-deficient mice develop normally but show increased anxiety in association with morphological abnormalities in the length and complexity of neurites of neurons in the basolateral amygdala. However, no behavioral abnormalities were detected in hippocampal-dependent memory tasks, which correlated with lack of any obvious hippocampal morphological deficits or alterations in basal synaptic transmission and long-term potentiation. *In vivo* reduction of TrkB signaling by removal of one BDNF allele could be partially rescued by TrkB.T1 deletion, which was revealed by an amelioration of the enhanced aggression and weight gain associated with BDNF haploinsufficiency. Our results suggest that, at the physiological level, TrkB.T1 receptors are important regulators of TrkB.FL signaling *in vivo*. Moreover, TrkB.T1 selectively affects dendrite complexity of certain neuronal populations.

Key words: **TrkB.T1; BDNF; mouse; anxiety; neurites; amygdala**

Introduction

The neurotrophins NGF, BDNF, NT-3, and NT-4 are key regulators of the development and function of the mammalian nervous system (Bibel and Barde, 2000; Huang and Reichardt, 2001; Chao et al., 2006). Some of their prominent roles include regulation of cell survival and cell death, modulation of synaptic transmission, neurite outgrowth, and branching (Snider, 1994; Cellerino and Maffei, 1996; Tessarollo, 1998; McAllister et al., 1999; Hempstead, 2002; Lu et al., 2005). Neurotrophin functions are mediated by three type of receptors: the Trk tyrosine kinase receptors; p75, a member of the tumor necrosis factor receptor superfamily; and sortilin, a Vps10p domain-containing transmembrane protein (Bothwell, 1995; Chao and Hempstead, 1995; Friedman and Greene, 1999; Kaplan and Miller, 2000; Nykjaer et al., 2004). The existence of several Trk receptor isoforms, such as the full-length Trk tyrosine kinase receptors (TrkB.FL) and the truncated isoforms (TrkB.T1 and TrkC.T1), which lack intrinsic tyrosine kinase activity, suggests the presence of additional mechanisms to diversify neurotrophin-induced signaling (Klein et al., 1990; Middlemas et al., 1991; Tsoulfas et al., 1993; Valenzuela et al., 1993; Garner and Large, 1994). It has been reported that both truncated TrkB.T1 and TrkC.T1 are capable of signaling independently. However, the physiological significance of these truncated Trk receptor activated signaling pathways is still unknown (Baxter et al., 1997; Rose et al., 2003; Ohira et al., 2005; Esteban et al., 2006). Moreover, although a number of studies using overexpression of these receptors has suggested that both truncated TrkB and TrkC can inhibit full-length tyrosine kinase signaling through a dominant-negative mechanism, the relevance of this mechanism *in vivo* by the endogenous truncated Trk receptor is still unknown (Biffo et al., 1995; Eide et al., 1996; Palko et al., 1999; Dorsey et al., 2006). In addition, it is also unknown whether specific functions, such as control of dendritic morphology, which are affected by overexpression of truncated TrkB receptors, are regulated by this receptor isoform at the physiological level (Yacoubian and Lo, 2000).

TrkB.T1 is considered the prototype of truncated Trk receptors. It is dynamically upregulated during fetal development and becomes the predominant Trk receptor isoform in the adult animal (Allendoerfer et al., 1994; Escandón et al., 1994; Fryer et al., 1996). Yet, to date, little is known about its physiological function

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in vivo. To address this issue, we have taken advantage of a mouse model that specifically lacks the TrkB.T1 receptor isoform but retains normal spatiotemporal levels of the full-length kinase active TrkB (Dorsey et al., 2006). Although TrkB.T1-deficient mice do not show any overt phenotype, we found that they are more anxious than their control littermates and have morphological changes in the length and complexity of neurites of the basolateral amygdala neurons. Moreover, although loss of TrkB.T1 overall does not affect normal brain development or function, we found that its reduction can improve deficiencies associated with BDNF haploinsufficiency *in vivo*.

Materials and Methods

Animals

TrkB.T1-deficient mice were generated as described previously and backcrossed 10–12 generations to C57BL/6 mice to provide a sufficiently homogeneous genetic background for testing (Dorsey et al., 2006). We used BDNF heterozygous mice backcrossed on a pure C57BL/6 background (Lyons et al., 1999) to obtain double mutant lines BDNF; TrkB.T1 to test for rescue of the *BDNF* haploinsufficiency phenotype. All mice were housed two to five per cage, unless otherwise stated, in a temperature- and humidity-controlled vivarium with water and food available *ad libitum* and maintained on a 12 h light/dark cycle. All animals were treated in compliance with the National Institutes of Health *Guide for Care and Use of Laboratory Animals* and approved by institutional Animal Care and Use Committees.

Behavioral analysis

To reduce experimental variability, male age-matched littermate pairs resulting from heterozygous crossings were used for all experiments. All behavioral measurements were performed by raters blind to genotype.

Weight analysis. Male mice of the indicated genotype were grouped housed and fed a standard chow diet containing 9% crude fat (PMI Nutrition). Weight was monitored monthly up to 7 months of age.

Resident-intruder assay (Lyons et al., 1999). For isolation-induced aggression, male resident test mice were single housed for at least 4 weeks. Cages were changed once per week but not during the week preceding testing. Aggressive behaviors in 3.5-month-old test mice were monitored during 5 min exposures to wild-type (WT) C57BL/6 male intruder mice that had been group housed (five per cage) and carefully matched with resident mice for body weight. Four test sessions were conducted (one trial per day). The latency to first biting attack and the total number of biting attacks were recorded from videotapes of each test session. For mice that failed to attack, the latency was scored as 5 min.

Open-field test. The open-field apparatus consisted of a white Plexiglas arena (40 × 40 × 49 cm) with a white floor divided into 12 equal quadrants. The arena was set up in a dim room under a digital camera connected to a video recorder and a computer under the control of the EthoVision tracking system (Noldus). A single mouse was placed into the center of the open-field arena, and its behavior was recorded over a 10 min session. Anxiety level was measured by the relative amount of exploration devoted to the center quadrants relative to those located adjacent to the walls of the arena. This was quantified by two indices: (1) percentage of time spent in the center quadrants and (2) percentage of entries into the center quadrants. An entry into a given quadrant was only registered if the center mass of the mouse crossed into the quadrant.

Elevated-plus maze. The elevated-plus maze was constructed of white Plexiglas and raised 70 cm above the floor. The apparatus consisted of two opposite enclosed arms with 14-cm-high opaque walls and two opposite open arms of the same size (30 × 5 cm). The arena was set up in a dim room under a digital camera, connected to a video recorder and a computer under the control of the EthoVision tracking system. A single testing session lasting 10 min was performed in a dark room. To begin a trial, the test animal was placed in the center of the plus maze facing an open arm, and its behavior was tracked for 10 min. The maze was cleaned with a 50% ethanol solution and dried after each trial to eliminate possible odor cues left by previous subjects. The number of entries into both the open and enclosed arms (an entry was scored when the center mass of the animal crossed into an arm), the time spent in those two areas, and the frequency of total crosses were recorded. Anxiety levels were measured by the relative amount of exploration devoted to the open arms relative to that to the enclosed arms. This was quantified by two indices: (1) percentage of time spent in the open arms and (2) percentage of entries into the open arms.

Fear conditioning. The conditioning apparatus consisted of a mouse shock chamber (Coulbourn Instruments) set up in a sound-attenuated box and scented with peppermint odor (0.1% peppermint). On day 1, the conditioning day, after a 2 min acclimation period to the conditioning chamber, mice received three conditioning trials consisting of a 30 s presentation of a 5 kHz, 70-dB tone [conditioning stimulus (CS)] that coterminated with a 0.7 mA foot shock delivered through the grid floor during the last 1.0 s of the tone. Each conditioning trial was separated by a 30 s intertrial interval (ITI). After conditioning, mice were returned to their home cages. Mice were videotaped during CS presentations for subsequent quantification of behavior. Time spent "freezing" before and during the presentation of the CS tone was measured during the CS presentation as well as during a 30 s baseline period before the first tone trial. This latter measure served as an assay for unconditioned effects of the CS on general activity levels. Memory for the context and the tone was evaluated on days 2 and 3 respectively (~24 and 48 h after conditioning). For the context test, mice were placed in the conditioning chamber and allowed to explore for 2 min, after which freezing to the context was assessed for the remaining 4.5 min. For the tone test, mice were placed in a novel chamber (circular in shape, with green walls, and scented with lemon odor). Mice were allowed to acclimate to the chamber for 2 min and were then presented with the CS (tone) on three consecutive trials (30 s, 5 kHz, 70 dB; ITI of 40 s). Freezing was evaluated during the 2 min acclimation period, during each presentation of the tone CS, and during the 40 s intertrial interval. After memory tests, animals were returned to their home cage and colony. Memory for either the context or tone CS was quantified by the percentage of time engaged in a fear-related behavior (freezing) during context testing or CS presentation.

Histology and morphological analyses

Rapid Golgi impregnation. Golgi impregnation of all brains was conducted using FD Rapid GolgiStain Kit (FD Neuro Technologies). Golgi–Cox (G–C) solution (mixture of A and B solutions from kit) was mixed a minimum of 12 h before use and stored in a dark place at room temperature. Care was taken during all steps to ensure that solutions did not come in contact with metal surfaces. After extraction from the skull, the brains were immersed in G–C solution in a glass bottle for 14 d at room temperature in a dark place (the G–C mixture was changed after the initial 12 h of impregnation). After the 14 d of incubation in G–C solution, the brains were transferred to solution C (10 ml/brain) and incubated for a minimum of 3 d at 4°C, again with the solutions having been changed after the initial 12 h. Brains were then embedded in a 3% agarose solution, blocked, and cut at room temperature on a vibratome (150 μm sections). Serial sections were immediately mounted onto 0.3% gelatin-coated slides. Once on the slides, and before complete drying of the tissue, the sections were brushed with solution C and allowed to air dry for 48 h. Slides were then immersed in ddH_20 three times for 5 min with gentle shaking, transferred into a solution of D plus E (from the Golgi kit) (25 ml of D, 25 ml of E, and 150 ml of dH_20) for 5–10 min at 4°C, and again rinsed three times (5 min) in ddH_20. Slides were then dehydrated through graded ethanols, cleared with Histoclear (three times for 5 min), and coverslipped with DPX mounting medium.

Golgi tracing: dentate gyrus and basolateral amygdala neuron inclusion criteria and analyses. Slides containing the Golgi-impregnated brain sections were coded before quantitative analysis to blind the experimenter to genotype; the code was not broken until the analysis was complete. Hippocampal dentate gyrus (DG) neurons were examined in the dorsal hippocampus. With respect to the basolateral amygdala, pyramidal-like neurons were analyzed using inclusion criteria established in previously published morphological studies (Vyas et al., 2002, 2004; Mitra et al., 2005). To be selected for analysis of dendritic arborization, Golgi-impregnated DG granule cells needed to satisfy the following criteria: (1)

isolated cell body with a clear relationship of the primary dendrite to the soma; (2) presence of untruncated dendrites and dark impregnation along the extent of all of the dendrites; and (3) relative isolation from neighboring impregnated cells that could interfere with the analysis. For each brain, 50 neurons from each region were selected. Cells were traced under 40× magnification using Neurolucida software (MicroBrightField). The morphological traits of cells (Sholl analysis and fractal dimension analysis) were analyzed using Neuroexplorer (MicroBrightField). Data were processed and analyzed statistically using Prism 4.0 (GraphPad Software).

Electrophysiology

After isofluorane anesthesia, decapitation, and removal of the brain, transverse hippocampal slices (350 μm thick) were obtained with a vibroslicer (Leica) in ice-cold artificial CSF (ACSF) containing the following (in mM): 125 NaCl, 1.25 KCl, 1 $CaCl_2$, 1.5 $MgCl_2$, 1.25 KH_2PO_4, 25 $NaHCO_3$, and 16 glucose, pH 7.4. Ten micromolar kynurenic acid was added to the sectioning solution to reduce excitotoxicity. Slices were incubated for 1 h at 32°C in a surface chamber filled with ACSF in which $CaCl_2$ was raised to 2.5 mM, and a gas mixture (95% O_2, 5% CO_2) was continuously bubbled. After the first hour, temperature was reduced to 28°C, and slices were kept in the same chamber until the transfer to the recording chamber. Slices were used within 10 h after sectioning. Extracellular field recording was performed in a submerged recording chamber. Slices were perfused with ACSF at 28°C at the rate of 2 ml/min. Two Teflon-coated concentric platinum–iridium electrodes were placed in the stratum radiatum in the CA1 area of the dorsal hippocampus, ~300–400 μm apart. Borosilicate glass recording electrodes were pulled (Sutter Instruments P90), ACSF filled to get 4–7 MΩ resistance, and placed in the apical dendritic region of CA1 pyramidal neurons evenly spaced with respect to the stimulating electrode. Field EPSP (fEPSP) was obtained by alternate stimulation of the two electrodes by activation of the Shaffer collaterals. One of the electrodes was used as a control electrode, whereas the other was used to deliver the conditioning protocol. An input–output curve was obtained independently for each of the stimulating electrodes by gradually increasing the stimulus intensity until fEPSP reached a plateau, after which the stimulus was reduced to obtain a fEPSP that was 50% of the maximum. Baseline recording was obtained by stimulating the slice every 20 s for at least 45 min. Once the baseline was stabilized to obtain long-term potentiation (LTP), two 250 ms, 100 Hz trains every 20 s were delivered to the conditioning electrode. The weaker conditioning protocol compared with the commonly used one of two 1 s, 100 Hz was used to avoid saturation of the LTP and allow to record an eventual increase of the response in the mutant mouse. Baseline recording was then resumed and followed for 1 h. Field potential was recorded (Multiclamp 700b; Molecular Devices), digitized (10 kHz Digidata 1324), low-pass filtered (3 kHz, eight-pole Bessel), and stored (Clampex 9.2; Molecular Devices). Signals were analyzed offline (Clampfit 9.2; Molecular Devices), and the size of the fEPSP was evaluated by measuring the initial slope of the signal expressed as percentage of the variation from the baseline value (average of 5 min before the conditioning protocol). Results were further analyzed with Igor Pro 6.01 (WaveMetrics). All data are reported as means ± SEs.

Statistics for behavior studies and morphological analyses

When two means were compared, statistical significance of their difference was calculated using nonpaired Student's *t* test. In multiple comparisons, data were analyzed by one-way ANOVA with a Bonferroni's *post hoc* test to determine statistical significance between genotypes. One-way ANOVA followed by Tukey's multiple comparison test was used for statistical analysis of weight and aggressive behavior.

Results

We have reported previously that TrkB.T1-deficient animals are viable, fertile, and do not display any overt phenotype (Dorsey et al., 2006). Targeting of the TrkB.T1 coding exon does not cause compensatory upregulation of other truncated TrkB receptor isoform (i.e., TrkB.T2) in neither neurons nor glia (Dorsey et al., 2006) (data not shown). In addition, we have not detected any change in the level of expression of either full-length or truncated TrkC receptors (data not shown), suggesting that possible abnormalities in this mouse model are caused by the specific deletion of TrkB.T1.

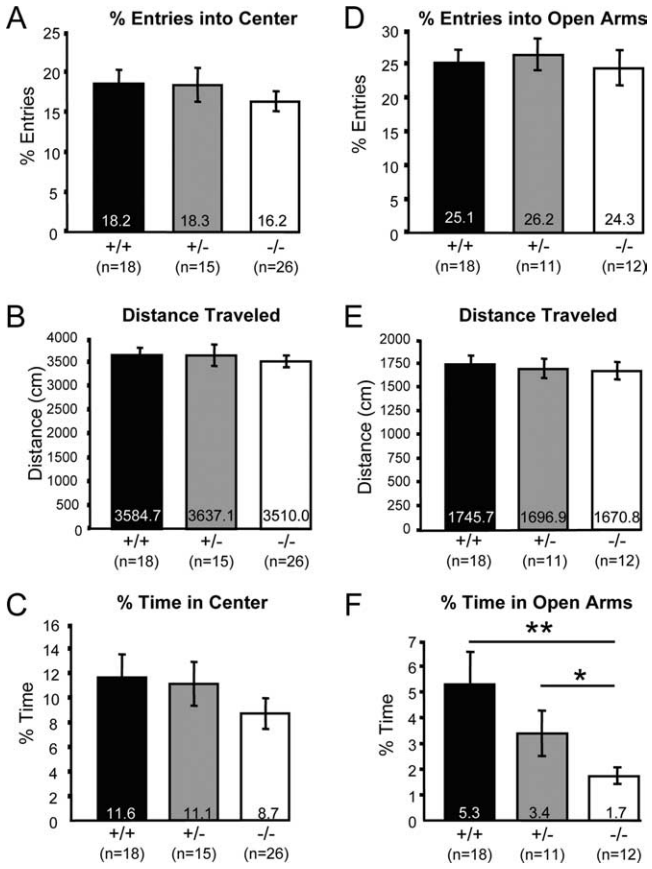

Figure 1. TrkB.T1-deficient animals have increased anxiety-like behavior. The behavior of +/+, +/−, and −/− *TrkB.T1* mice was analyzed using the open-field (***A–C***) and the elevated-plus maze (***D–F***) tests. Mouse performances were video recorded and analyzed blindly. For the open-field test, the number of mouse entries into the center (***A***), distance traveled (***B***), and time spent in the center of the arena (***C***) were determined. Anxiety level was measured by the relative amount of exploration devoted to the central quadrants relative to those located adjacent to the walls of the arena. For the elevated-plus maze test, the number of entries into the open arms (***D***), distance traveled (***E***), and time spent in the open arms (***F***) were scored. Anxiety was measured by the relative amount of exploration invested in the open arms relative to that of the enclosed arms. For each test, mouse behavior was followed for 10 min. $^*p < 0.05$; $^{**}p < 0.01$.

Because BDNF/TrkB-activated signaling pathways are critical for the control of a variety of developmental processes, including nervous system functions, we characterized this mutant to assess the role of TrkB.T1 in TrkB.FL signaling and in long-term organism homeostasis. Aging of mutant mice for over 2 years revealed that they have a normal lifespan compared with WT littermates (data not shown). Furthermore, they do not show changes in tumor development, suggesting that TrkB.T1 does not control cell proliferation per se (data not shown). We then investigated whether endogenous TrkB.T1 causes specific developmental deficits or whether it exerts inhibitory roles on TrkB.FL at the physiological level. These two aspects of the characterization of the TrkB.T1 mouse model are important because of the suggested intrinsic signaling role for TrkB.T1 (Baxter et al., 1997; Rose et al., 2003; Ohira et al., 2005) and because TrkB.T1 dominant-negative *in vivo* functions on TrkB.FL signaling have been demonstrated only in pathological or transgenic overexpression conditions

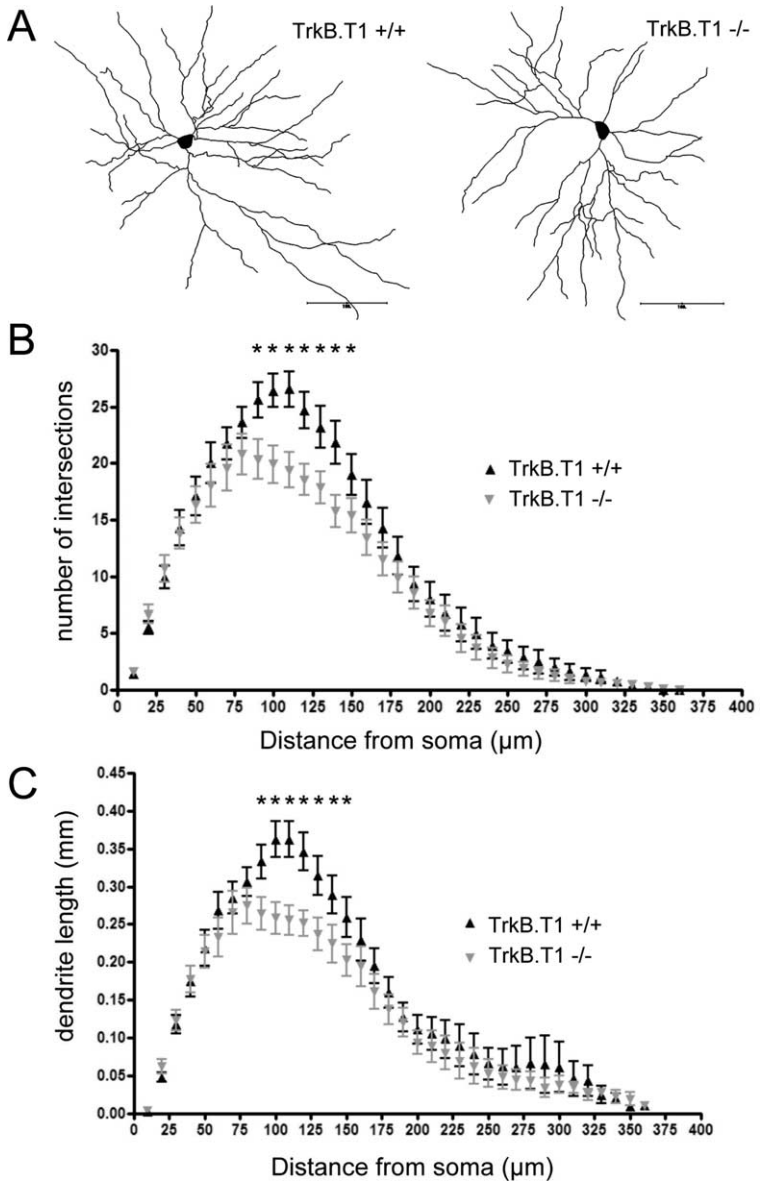

Figure 2. *TrkB.T1*$^{-/-}$ mice have decreased neuronal complexity in the amygdala. Morphological analysis of basolateral amygdala neurons visualized by the rapid Golgi impregnation method. ***A***, Neurolucida representation of a typical stained basolateral amygdala neuron from *TrkB.T1*$^{-/-}$ (right) and wild-type control (left) mouse brain sections. Sholl analysis of the number of dendrite intersections (***B***) and length of dendrites of amygdala neurons (***C***). Postnatal day 60 mice and 25 neurons per mouse were used in the analysis. All results are presented as means ± SEM determined from analyzing six mice per genotype. *$p < 0.01$.

(Saarelainen et al., 2000a,b; Dorsey et al., 2006). No direct *in vivo* role for endogenous TrkB.T1 has been demonstrated to date.

TrkB.T1-deficient mice have increased anxiety and basolateral amygdala dendrite abnormalities

To address the role of TrkB.T1 in neuronal functions, we performed behavioral analyses on TrkB.T1-deficient mice to assess phenotypes that are affected by BDNF/TrkB signaling such as anxiety, learning and memory, and aggression.

Mice were first subjected to the open-field test to investigate whether there was any difference in basal locomotor activity and the time spent in the center versus the edges of the arena. No significant differences were observed in either parameter analyzed, suggesting that TrkB.T1 mutant mice do not have any major impairment in this paradigm (Fig. 1*A–C*). However, we did notice a trend toward mutant mice spending less time in the center of the arena and making fewer entries into the middle, suggesting that TrkB.T1 deficiency may cause increased anxiety (Fig. 1*A*, *C*). We then used the elevated-plus maze, an assay that is more sensitive in detecting anxiety-related phenotypes. Although *TrkB.T1*$^{-/-}$ mice entered the open arms as many times as their control littermates, they did spend significantly less time in the open arms, a behavior that is consistent with an increased anxiety phenotype (Fig. 1*D–F*). TrkB.T1 is highly expressed in the hippocampus, a region associated with learning and memory functions. Moreover, impairments in BDNF signaling have been shown to affect hippocampal-dependent memory functions in both humans and rodents (Egan et al., 2003; Hariri et al., 2003; Chen et al., 2006). Thus, we subjected TrkB.T1 mutant mice to a contextual fear-conditioning test, a hippocampus- and amygdala-dependent memory task. We found no differences in the percentage of freezing to context (52.8 ± 7.9% WT, $n = 10$; 46.2 ± 6.3% *TrkB.T1*$^{-/-}$, $n = 9$) and to cue (68.9 ± 3.5% WT, $n = 10$; 73.7 ± 1.6% *TrkB.T1*$^{-/-}$, $n = 9$) between WT or TrkB.T1 mutant animals, suggesting that there are no impairments in either associative learning or hippocampal-dependent encoding of environmental cues. The specific alteration in anxiety levels and the lack of deficits in memory functions prompted us to investigate whether TrkB.T1 deletion caused any anatomical deficit in the amygdala, a brain region known to control anxiety in mammals. We used Golgi staining to visualize individual basolateral amygdala neurons (Fig. 2*A*). At 8 weeks of age, there was no difference in cell soma area between TrkB.T1 mutant mice and their WT controls (data not shown). Next, we analyzed dendritic complexity in these same neurons. Sholl analysis revealed a decrease in dendritic arbor complexity at 90 μm and greater distances from the soma in TrkB.T1 mutant neurons (Fig. 2*B*). In addition, we also observed a decrease in dendritic length (Fig. 2*C*). These observations, in correlation with the elevated-plus maze results, suggest that the reduced morphological complexity of *TrkB.T1*$^{-/-}$ neurons in the basolateral amygdala can be in part responsible for the increased anxiety of TrkB.T1 mutant mice. When the morphology of neurons in the dentate gyrus of the hippocampus was examined, no significant differences between genotypes were detected in cell soma size (data not shown), dendrite length (data not shown), or dendritic complexity by Sholl analysis (Fig. 3). There was a trend toward decreased dentate gyrus dendritic arbor complexity at 150 μm and greater distances in TrkB.T1 mutant mice, but it was not statistically significant (Fig. 3*B*). This result correlates with the behavioral analysis, because TrkB.T1 mutant mice showed no deficits

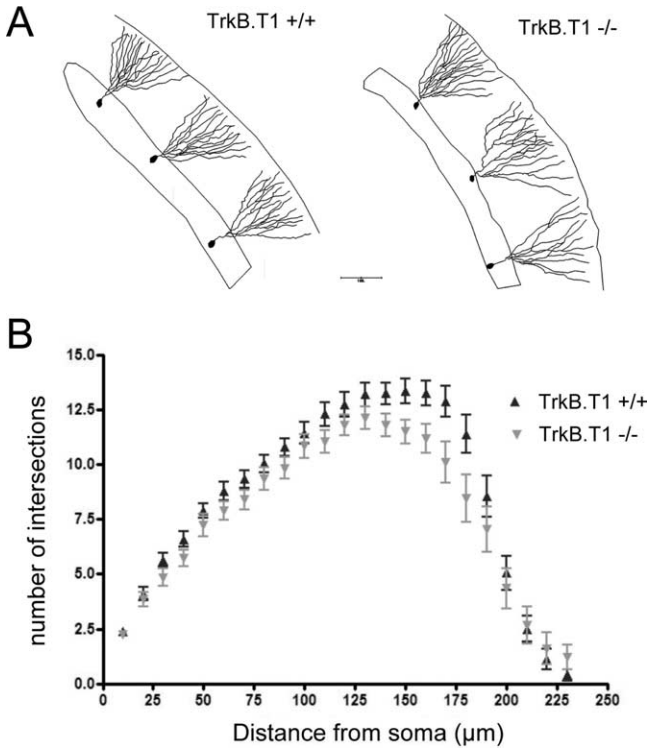

Figure 3. Loss of TrkB.T1 does not affect hippocampus neurite morphology. ***A***, Representative examples of Golgi-stained dentate gyrus neurons from postnatal day 60 wild-type (+/+; left) and TrkB.T1-deficient (−/−; right) mice. Sholl analyses of the number of intersections (***B***) in hippocampal dentate gyrus neurons. All results are presented as means ± SEM determined by analysis of six mice per genotype and 25 neurons per mouse. $^{*}p < 0.01$.

in learning and memory performance in the hippocampal-dependent contextual fear-conditioning paradigm.

TrkB.T1 does not affect hippocampal basal synaptic transmission and LTP

To further characterize whether the TrkB.T1 deletion may result in deficits that are not immediately apparent by behavioral or morphological characterization, we decided to analyze the basal synaptic transmission and LTP in TrkB.T1-deficient mice. We reasoned that a small deficit in LTP may not cause a short-term anatomical or behavioral abnormality but could have long-term effects on the animal's memory functions. We found that LTP in the CA1 area of the dorsal hippocampus at 1 h after the conditioning in WT animal (141.75 ± 6.25%, $n = 16$) was similar to that found in TrkB.T1-deficient mice (128.14 ± 9.84%, $n = 10$), suggesting that loss of TrkB.T1 does not affect this aspect of hippocampal synaptic plasticity (Fig. 4). Furthermore, the basal synaptic transmission appeared to be normal, as assessed by the input–output curves (stimulus intensity vs fEPSP slope) from the Schaffer collateral–CA1 region. Again, these results are consistent with the normal learning and memory function observed in the behavioral studies and the lack of abnormalities in the morphology of DG neurons in TrkB.T1 mutant animals.

TrkB.T1 deletion partially rescues BDNF haploinsufficiency

We and others have reported previously that *BDNF* heterozygous mice exhibit enhanced aggression as well as increased food consumption that leads to obesity (Lyons et al., 1999; Kernie et al., 2000; Rios et al., 2001; Coppola and Tessarollo, 2004). Because the severity of the phenotypes caused by BDNF/TrkB deficiency parallels the level of loss of this signaling pathway (Lyons et al., 1999; Rios et al., 2001; Xu et al., 2003), we reasoned that even minimal increases in BDNF/TrkB signaling could have an impact on the degree of obesity and aggression caused by deletion of a single BDNF allele. Indeed, introducing the TrkB.T1 mutation into a $BDNF^{+/-}$ background partially rescues both phenotypes, consistent with an inhibitory role for TrkB.T1 on TrkB/BDNF signaling (Fig. 5). Specifically, over a 7 month period, $BDNF^{+/-}$; $TrkBT1^{-/-}$ mice gained significantly less weight than $BDNF^{+/-}$ mice. $BDNF^{+/-}$;$TrkB.T1^{+/-}$ mice were not significantly different weight-wise early in life. However, over time, their weight stabilized, achieving a lower final weight than $BDNF^{+/-}$ mice (Fig. 5*A*).

When applying the resident-intruder paradigm, both $BDNF^{+/-}$;$TrkB.T1^{+/-}$ and $BDNF^{+/-}$;$TrkBT1^{-/-}$ mice showed a significant decrease in aggressive behavior as measured by the latency to first attack and the total number of biting attacks during 5 min exposures to an intruder mouse (Fig. 5*B*,*C*). Importantly, the partial rescue observed in both $BDNF^{+/-}$;$TrkB.T1^{+/-}$ and $BDNF^{+/-}$;$TrkBT1^{-/-}$ mice suggests that the phenotypic changes are not caused by intrinsic physiological alterations attributable to the complete loss of TrkB.T1 but rather by a reduced dominant-negative effect on TrkB.FL or a decrease in BDNF sequestration, resulting in the potentiation of TrkB.FL signaling.

Discussion

The physiological role of TrkB.T1 is still unknown. Here we examined the consequences of TrkB.T1 deletion in mouse development as well as the role of the endogenous TrkB.T1 receptor on BDNF signaling *in vivo*. We found that loss of TrkB.T1 led to increased anxiety-related behavior that is associated with structural alterations in neurites of neurons of the amygdala. Moreover, we show that reducing TrkB.T1 levels *in vivo* partially rescues the phenotypes caused by loss of one BDNF allele.

Truncated TrkB.T1 receptors were first described almost 20 years ago, but to date very little is known about their physiological role in neurotrophin signaling and development. One of the major obstacles in identifying these functions has been the absence of a suitable animal model lacking only this receptor isoform. Previous animal models have been generated targeting either the full-length isoform or all TrkB isoforms (Klein et al., 1993; Rohrer et al., 1999). TrkB.FL receptor exerts strong prosurvival functions in neurons, and, consequently, its deletion causes extreme phenotypes, which have made it impossible to evaluate any long-term functional roles the truncated TrkB isoform might have. Recently, we targeted the TrkB locus to specifically delete the TrkB.T1 isoform without affecting the level or the spatiotemporal pattern of expression of TrkB.FL (Dorsey et al., 2006). These animals are viable and fertile, and no obvious phenotype has been detected by simple observation. The lack of strong developmental phenotypes in our model supports the idea that the predominant role of TrkB.T1 is not to support neuronal survival. Instead, it might be involved in the regulation of BDNF signaling and in the differentiation and/or function of neurons. Alternatively, it suggests that other truncated TrkB (e.g., TrkB.T2) or TrkC receptors may compensate for TrkB.T1 deficiency. However, we did not find any upregulation of other truncated TrkB or TrkC receptor isoform in either neurons nor glia lacking TrkB.T1. Moreover, truncated TrkC receptors are present at a significantly lower level than TrkB.T1, suggesting that, at least at the expression level, other truncated Trk receptors may not be able to compensate for the loss of the most expressed of all truncated Trk receptors (Dorsey et al., 2006, and data not shown).

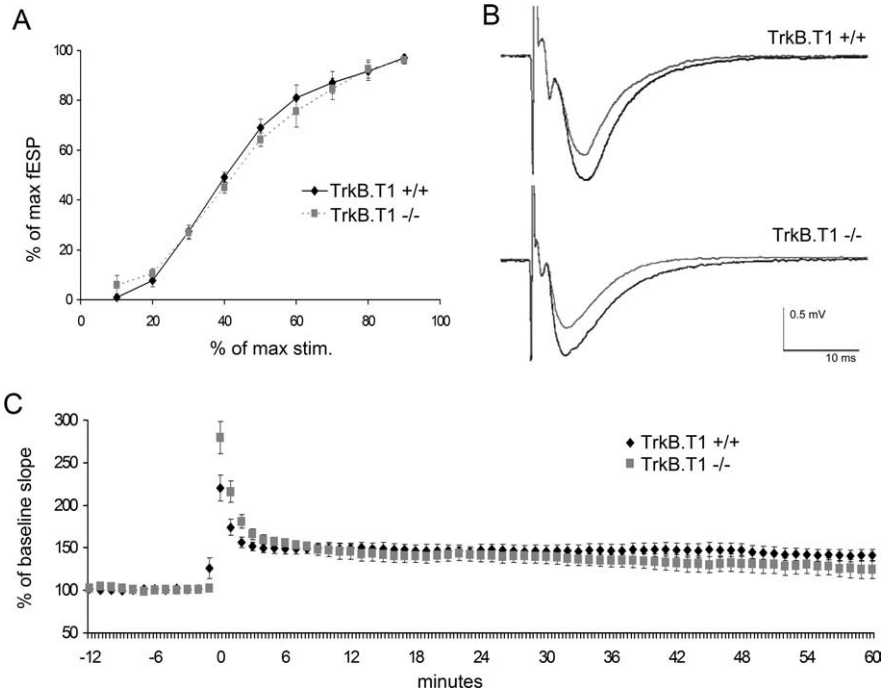

Figure 4. TrkB.T1 does not affect hippocampal basal synaptic transmission and LTP. ***A***, Input–output curve: initial slope of fEPSPs obtained with increased stimulus intensity were plotted versus the response normalized to the maximal response obtained ($n = 5$ per genotype). ***B***, fEPSP recorded in wild-type and mutant TrkB.T1 mice. The average of five traces obtained before (gray) and 1 h after (black) the LTP conditioning protocol (2 times at 250 ms, 100 Hz train) are represented. ***C***, Time course of initial slope of fEPSP before and after LTP induction in wild-type (black diamonds; $n = 12$) and *TrkB.T1*$^{-/-}$ (gray squares; $n = 11$) mice.

A role for TrkB.T1 in regulating BDNF signaling was first proposed when it was cloned, and such a function has been supported by its pattern of expression and a number of *in vitro* and *in vivo* overexpression experiments (Klein et al., 1990; Middlemas et al., 1991; Biffo et al., 1995; Eide et al., 1996; Saarelainen et al., 2000b; Haapasalo et al., 2001). However, whether physiological levels of this receptor isoform could exert such activity has never been definitively proven. Our study, by showing partial rescue of BDNF haploinsufficiency by TrkB.T1 deletion proves that physiologically TrkB.T1 indeed limits BDNF signaling *in vivo* (Fig. 5). A key question is why would an organism require a negative modulator of BDNF signaling such as TrkB.T1? A number of studies have suggested that excessive BDNF is involved in the pathogenesis of epilepsy, mania, and autism (for review, see Tsai, 2007). Moreover, it has been shown that TrkB.FL is required for epileptogenesis in the kindling model and that zinc, a metal abundantly present in the brain, can transactivate synaptic TrkB by a neuronal activity-regulated mechanism (He et al., 2004; Huang et al., 2008). Thus, although TrkB.FL signaling is important for synaptic plasticity, it appears that excessive activation of this receptor could be one of the causes leading to hyperexcitability of specific brain areas, which in turn could cause epilepsy. The finding that physiological TrkB.T1 limits BDNF signaling *in vivo* suggests that TrkB.T1 may be part of a mechanism critical in preventing pathological activation of the TrkB.FL. It will be of interest to investigate whether TrkB.T1 may represent an important buffer to prevent overactivation of TrkB.FL during neuronal activity.

Alternatively, the primary function of TrkB.T1 could be the modulation of other cellular functions independent of the TrkB kinase receptor (Baxter et al., 1997; Rose et al., 2003; Ohira et al., 2005). For example, TrkB.T1 has been reported to regulate astrocytic morphology by directly interacting with Rho GDP dissociation inhibitor 1 and modulate calcium release from intracellular stores in astrocytes (Rose et al., 2003; Ohira et al., 2005). However, the lack of a more dramatic phenotype in this model also indicates that TrkB.T1 does not have a critical widespread function in CNS, as might be suggested by its potential role in astroglia calcium homeostasis (Reichardt, 2003).

TrkB.FL and TrkB.T1 expression are tightly regulated during development. Although TrkB.FL is the highest expressed isoform in early CNS development, TrkB.T1 is dramatically upregulated during postnatal brain development (Allendoerfer et al., 1994; Escandón et al., 1994; Fryer et al., 1996). The reason for this tight regulation of TrkB receptor isoform expression is unknown. In addition to the above-discussed role in the control of TrkB.FL activation, it has been suggested that TrkB.T1 and TrkB.FL can regulate distinct modes of dendritic growth in visual cortical neurons. Specifically, TrkB.FL promotes the addition of short branches in dendritic regions proximal to the cell body, whereas TrkB.T1 induces the extension of dendrites in regions more distal to the soma. These data suggest that expression of the correct set of TrkB isoforms is essential for normal dendritic development (Yacoubian and Lo, 2000). Indeed, we found that TrkB.T1 deficiency does affect neurite complexity, as well as dendrite length of neurons of the amygdala. Although we cannot assess whether this effect is caused by a dominant-negative inhibition of TrkB.FL or by a different mechanism, these data provide definitive evidence that, in certain neuronal populations, physiological TrkB.T1 is important in regulating neuronal branching. This effect is not widespread because, in contrast with the amygdala, our behavioral and structural analysis of the hippocampus has not shown any change so far, suggesting that there are different regional TrkB.T1 requirements during neuronal development.

TrkB.T1 is also present at cortical glutamatergic synapses together with TrkB.FL, suggesting that it may play a role in synaptic plasticity (Gomes et al., 2006). Surprisingly, we have so far failed to detect any clear electrophysiological abnormality in the hippocampus, a region whose neurons express both full-length and TrkB.T1 receptors. Lack of an effect on induction or maintenance of LTP has been reported also in transgenic mice overexpressing TrkB.T1 in the cortex and the hippocampus, suggesting that TrkB.T1 is not limiting for the induction of LTP (Saarelainen et al., 2000a). A previous study had shown LTP inhibition in hippocampal slices overexpressing TrkB.T1 delivered by adenoviral infection, but it has been suggested that different levels of expression at synapses were responsible for the discrepancy (Li et al., 1998; Saarelainen et al., 2000a). Nevertheless, our data strongly indicates that, at least in young animals, endogenous TrkB.T1 is not limiting the induction of LTP.

In conclusion, we have shown that truncated TrkB.T1 receptor is indeed an important regulator of BDNF signaling *in vivo*, it is involved in the control of complex behaviors, and it affects

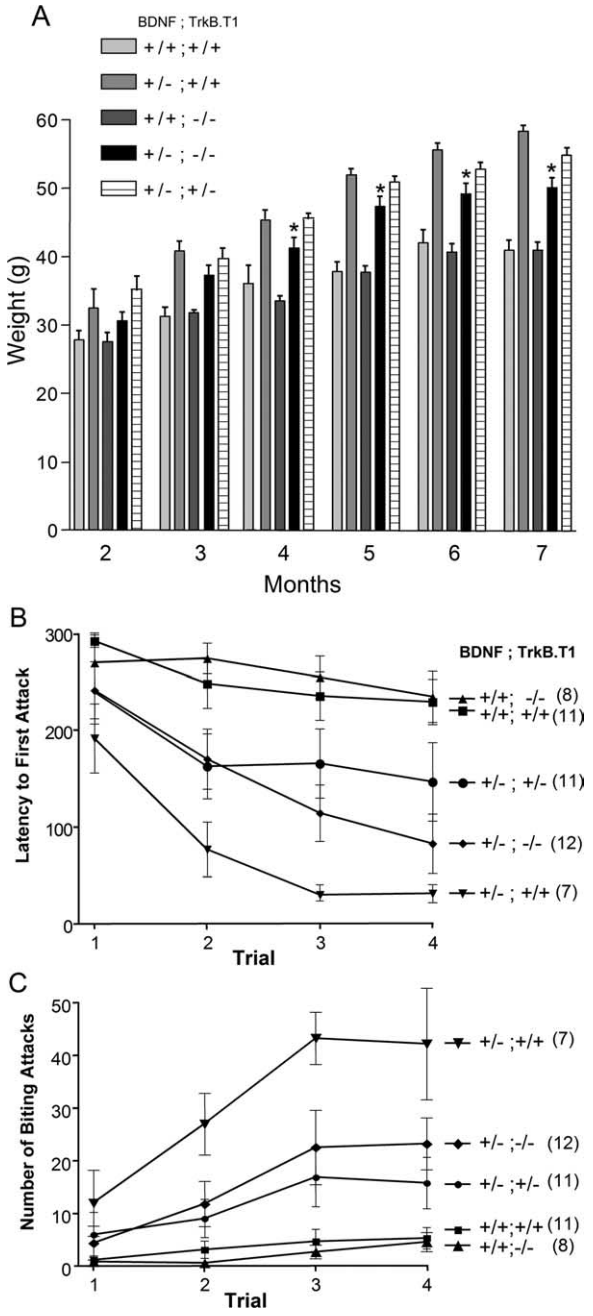

Figure 5. TrkB.T1 deletion partially rescues the obesity and the aggressive phenotype caused by partial loss of BDNF. ***A***, Weight analysis of wild-type, *TrkB.T1*$^{-/-}$, *TrkB.T1*$^{-/-}$;*BDNF*$^{+/-}$, *TrkB.T1*$^{+/-}$;*BDNF*$^{+/-}$, and *BDNF*$^{+/-}$ mice. Mice had *ad libitum* access to food and water. The weight of the indicated mouse groups was monitored once a month until mice reached an age of 7 months. Bars indicate means ± SEM. Each group comprised a minimum of eight mice. At 4 months of age, one-way ANOVA statistical analysis followed by Tukey's multiple comparison test shows that the weight of *BDNF*$^{+/-}$;*TrkB.T1*$^{-/-}$ is significantly different from that of *BDNF*$^{+/-}$ (*$p < 0.05$) mice but not from that of wild-type animals. At 5, 6, and 7 months, the weights of *BDNF*$^{+/-}$;*TrkB.T1*$^{-/-}$ are significantly different from both wild-type and *BDNF*$^{+/-}$ mice (*$p < 0.05$). ***B***, ***C***, Aggressive behavior was assessed for each of the above-indicated genotype using the resident/intruder paradigm. The latency to first attack (***B***) was measured on 4 consecutive days (1 session of 5 min a day) for each genotype using an age- and weight-matched wild-type intruder mouse. The number of mice for each group is indicated in parentheses. Means ± SEM bars are indicated. Latency to first attack in trial 3 as analyzed by one-way ANOVA and Tukey's multiple comparison test reveals a significant difference between *BDNF*$^{+/-}$ and *BDNF*$^{+/-}$;*TrkB.T1*$^{+/-}$ ($p < 0.05$) but not between *BDNF*$^{+/+}$;*TrkB.T1*$^{+/+}$ and *BDNF*$^{+/-}$;*TrkB.T1*$^{+/-}$. ***C***, Number of biting attacks over a period of 5 min. The number of biting attacks by *BDNF*$^{+/-}$ mice in trials 2–4 is significantly different ($p < 0.05$) from that of *BDNF*$^{+/-}$;*TrkB.T1*$^{+/-}$ but not of *BDNF*$^{+/-}$;*TrkB.T1*$^{-/-}$ mice.

neurite complexity in the amygdala. Additional analysis of aged animals and the use of paradigms to challenge these mutants will help to shed additional light into other physiological roles of TrkB.T1, the highest expressed TrkB isoform in the mature mammalian brain.

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