TrkB Regulates Hippocampal Neurogenesis and Governs Sensitivity to Antidepressive Treatment

Yun Li,1 Bryan W. Luikart,1 Shari Birnbaum,2 Jian Chen,1 Chang-Hyuk Kwon,1 Steven G. Kernie,1,3 Rhonda Bassel-Duby,4 and Luis F. Parada1,*

1Department of Developmental Biology, Kent Waldrep Center for Basic Research on Nerve Growth and Regeneration
2Department of Psychiatry
3Department of Pediatrics
4Department of Molecular Biology
University of Texas Southwestern Medical Center, Dallas, TX 75390-9133, USA
*Correspondence: luis.parada@utsouthwestern.edu
DOI 10.1016/j.neuron.2008.06.023

SUMMARY

Adult hippocampal neurogenesis is stimulated by chronic administration of antidepressants (ADs) and by voluntary exercise. Neural progenitor cells (NPCs) in the dentate gyrus (DG) that are capable of continuous proliferation and neuronal differentiation are the source of such structural plasticity. Here we report that mice lacking the receptor tyrosine kinase TrkB in hippocampal NPCs have impaired proliferation and neurogenesis. When exposed to chronic ADs or wheel-running, no increase in proliferation or neurogenesis is observed. Ablation of TrkB also renders these mice behaviorally insensitive to antidepressive treatment in depression- and anxiety-like paradigms. In contrast, mice lacking TrkB only in differentiated DG neurons display typical neurogenesis and respond normally to chronic ADs. Thus, our data establish an essential cell-autonomous role for TrkB in regulating hippocampal neurogenesis and behavioral sensitivity to antidepressive treatments, and support the notion that impairment of the neurogenic niche is an etiological factor for refractory responses to an antidepressive regimen.

INTRODUCTION

Depression is a significant public health problem due to both its high prevalence and its devastating impact on individuals and society. Despite much excitement generated by recent advances in the knowledge of brain development and function, the mechanisms underlying the pathogenesis of depression, as well as its amelioration by antidepressant (AD) treatment, remain poorly understood (Manji et al., 2001; Nestler et al., 2002). Clinical studies demonstrate that 40% of depressed patients fail to respond to first-line ADs and 70% do not undergo full remission (Fagiolini and Kupfer, 2003; Thase and Rush, 1995). Such a variable range of effectiveness underscores the heterogeneity of the illness and the urgent need for thorough delineation of the cellular and molecular process involved in the action of ADs.

As predicted by their pharmacological characteristics, most ADs appear to act by increasing the activity of serotonergic and noradrenergic circuits (Morilak and Frazer, 2004). However, these medications usually require chronic administration for weeks to months before clinically appreciable effects are achieved. This represents an extended delay compared with the rapid increase of serotonin and noradrenaline elicited by these drugs (Frazer, 1997). Therefore, gradual yet essential changes to the brain that occur during the period of delay appear to be required for the response to ADs. Animal studies indicate one such delayed response is the increased production of new neurons in the dentate gyrus (DG) (Malberg et al., 2000; Perera et al., 2007). Physical activity such as running, which is considered beneficial for mental health (Salmon, 2001), also induces increased production of neurons (van Praag et al., 1995). Studies in rodents have shown that upon exposure to chronic ADs or exercise, neural progenitor cells (NPCs) in the subgranular zone (SGZ) of the DG undergo enhanced proliferation, which drives the increase in neurogenesis (Encinas et al., 2006). Although the functional significance remains to be proven, the positive association of neurogenesis with ADs and exercise, as well as other beneficial stimulations such as diet, learning, and environmental enrichment (Alvarez-Buylla, 1992; Kempermann et al., 1997; Lee et al., 2000), implicates it as a physiologically relevant phenomenon. Indeed, irradiation-mediated ablation of proliferating cells in the hippocampus compromises the ability of certain strains of rodents to display a behavioral response to ADs, suggesting that neurogenesis is an intrinsic requirement (Airan et al., 2007; Hollick et al., 2008; Santarelli et al., 2003; Wang et al., 2008a). The genetic mechanism underlying this observation remains unclear. Coinciding with the elevation of neurogenesis, chronic AD exposure elicits a variety of molecular adaptations (Tardito et al., 2006). The mechanisms that link these molecular changes to the biological effects of ADs are only beginning to be unveiled. One of the molecules implicated is brain-derived neurotrophic factor (BDNF), whose level in the hippocampus is increased by chronic, but not acute, AD exposure (Nibuya et al., 1995). Infusion of BDNF into the DG produces AD-like responses in several behavioral paradigms (Shirayama et al., 2003). In humans, a single nucleotide
polymorphism of the BDNF gene (val66met) that interferes with the activity-dependent secretion of BDNF protein has been associated with cognitive and structural abnormalities of the CNS (Egan et al., 2003; Pezawas et al., 2004), though consensus is lacking on its influence on susceptibility to mood disorders (Choi et al., 2006; Jiang et al., 2005; Schule et al., 2006). Interestingly, recent generation of mutant mice carrying the same polymorphism indicates a causative link between the genetic change and elevated anxiety-like behaviors that are not normalized by ADs (Chen et al., 2006). These observations, coupled with evidence that exogenous BDNF promotes proliferation of hippocampal NPCs (Scharfman et al., 2005), suggest an important role for BDNF in mediating the biological response to chronic AD treatments (Wang et al., 2008b). Whether BDNF acts directly on NPCs in vivo, and whether its effects on NPCs then contribute to the overall influence of BDNF on AD response, is unresolved.

In the present study, we conditionally ablated the gene encoding TrkB, the high-affinity receptor for BDNF, in a regional- and cell-type-specific manner. We show that NPC deletion of TrkB, either in embryos or in the adult, results in impairment of hippocampal neurogenesis and prevents behavioral improvements induced by chronic AD administration or wheel-running. In contrast, deleting TrkB in differentiated neurons of the same brain regions does not affect neurogenesis or the behavioral responses to ADs. Our findings provide genetic evidence of a functional, cell-autonomous requirement of TrkB in the neurogenic and behavioral responses to antidepressive treatments. Furthermore, our results support the notion that DG NPCs are a required component in the amelioration of depression (Zhao et al., 2008).

RESULTS

TrkB Governs Sensitivity to Antidepressants

TrkB Is Expressed by Hippocampal NPCs

We and others have previously demonstrated the presence of TrkB transcripts and protein in the hippocampus (Klein et al., 1990; Zhou et al., 1993). To determine whether transcripts are present in the neurogenic zone, we first examined TrkB expression in the postnatal and adult DG. In situ hybridization analysis showed prominent expression of both BDNF and TrkB in the granular layer and SGZ of the DG (Figure 1A). In particular, TrkB mRNA, represented by silver bromide grains, was detectable throughout the cellular layers of the DG (Figure 1A, right panel). We further analyzed the distribution of TrkB protein in the adult dentate gyrus (DG). In the high-magnification image of the DG (Figure 1A, right panel), note the distribution of silver grains (black spheres) in all cells. Scale bars, 1 mm (low-magnification) and 10 μm (high-magnification). GL, granular layer; SGZ, subgranular zone.

(B) Confocal image of the DG of an adult Nestin-GFP transgenic mouse, coimmunostained for GFP (green), NeuroD (red), and NeuN (blue). GFP expression was restricted to NPCs and did not colocalize with immature (NeuroD-positive) or mature (NeuN-positive) neurons. Insert shows a DG-derived neurosphere that expresses GFP. Scale bars, 10 μm.

(C) RT-PCR detection of TrkB and BDNF transcripts in FACS-sorted Nestin-GFP-positive cells and DG-derived neurospheres. NS, neurosphere.

(D) Immunostaining for TrkB (green) on adult DG sections from wild-type and TrkB<sup>KO</sup> mice (left panels). Costaining for TrkB (green) and Ki67 (red) or Doublecortin (red) demonstrated colocalization of TrkB with proliferating (Ki67-positive) and differentiating (Doublecortin-positive) cells. Dcx, Doublecortin; WT, wild-type. Scale bars, 10 μm and 5 μm.

TrkB Is Expressed by Hippocampal NPCs

We and others have previously demonstrated the presence of TrkB transcripts and protein in the hippocampus (Klein et al., 1990; Zhou et al., 1993). To determine whether transcripts are present in the neurogenic zone, we first examined TrkB expression in the postnatal and adult DG. In situ hybridization analysis showed prominent expression of both BDNF and TrkB in the granular layer and SGZ of the DG (Figure 1A). In particular, TrkB mRNA, represented by silver bromide grains, was detectable throughout the cellular layers of the DG (Figure 1A, right panel). To further examine whether the DG NPCs express TrkB, we utilized Nestin-GFP transgenic mice, in which GFP expression is confined to NPCs (Figure 1B) (Yu et al., 2005). Using fluorescent-activated cell sorting (FACS), GFP-positive cells from the DG of transgenic mice at various ages were isolated, and analysis of these cells by RT-PCR demonstrated the presence of NPC markers and the absence of markers from the differentiated lineages (Figure S1 available online). TrkB and BDNF mRNAs were detected in the GFP-positive cells at all ages tested (postnatal day [P] 2, 15 and 60, n = 3 animals for each, Figure 1C). Similarly, TrkB and BDNF mRNAs were also detected in neurospheres derived from the DG of adult wild-type mice (n = 3, Figure 1C). We further analyzed the distribution of TrkB protein in the progenitor population by coimmunostaining. Consistent with its mRNA distribution, TrkB protein was detected in all cells. Scale bars, 10 μm and 5 μm.
Neuron

TrkB Governs Sensitivity to Antidepressants

Figure 2. hGFAP-Cre, but Not Syn-Cre, Mediated Recombination in Hippocampal NPCs

(A) X-gal staining on DG sections of R26hGFAP and R26Syn mice at P10 and P60. Higher magnification views of the boxed areas are shown in the right panels, in which black lines outline the granular layer, while the red double arrow highlights the SGZ and inner granular layer where recombination was spared. Scale bars, 100 μm.

(B) Schematic diagram of the procedure to generate neurospheres from adult DG.

(C) X-gal staining on primary neurospheres derived from the DG of adult control, R26hGFAP, and R26Syn mice. Blue staining in the neurosphere from R26hGFAP mice indicated the occurrence of recombination. Scale bar, 100 μm.

(D) Western blots of lysates from the hippocampus of control and TrkBhGFAP mice, probed for TrkB and actin. Note the absence of TrkB in the TrkBhGFAP mice.

(E) RT-PCR detection of TrkB and G3PDH transcripts in FACS-isolated Nestin-GFP-positive cells from the DG of control and TrkBhGFAP mice. Note the absence of TrkB in the TrkBhGFAP mice.

hGFAP-Cre Activity in Hippocampal Progenitor Cells

Germline homozygous TrkB knockout animals die shortly after birth. To investigate the role of TrkB in postnatal stages, conditional knockout animals were generated by crossing mice harboring the TrkB flox alleles to transgenic mice expressing the Cre recombinase either under the human GFAP (hGFAP) promoter or the Synapsin I (Syn) promoter (He et al., 2004; Luikart et al., 2005). In both the hGFAP-Cre and the Syn-Cre transgenic animals, the pattern of Cre expression allows recombination throughout the forebrain, including cerebral cortex, hippocampus, and olfactory bulb. In other regions of the brain, such as the midbrain (dopaminergic neurons) and brainstem (serotonergic neurons), Cre expression is minimal (Figure S4 and not shown). When these transgenic animals were interbred with Ros26stop-lacZ reporter mice (R26) (Soriano, 1999) and analyzed at the age of 2 months, a majority of the neurons in the aforementioned regions appeared to express functional beta-galactosidase (β-gal) (Figure 2A and not shown). However, when such animals were analyzed at a younger age (P10), a distinct anatomical difference in the recombination patterns of the two Cre lines was observed in the DG. In the R26hGFAP animal most cells throughout the DG expressed β-gal, whereas in the R26Syn animal, β-gal expression was confined to the outer layers of the DG, while the SGZ and inner granule layer, where NPCs and immature neurons reside, were essentially spared of recombination (Figure 2A).

We next enriched NPCs from 2-month-old mice by culturing primary cells from the DG in neurosphere-forming media. We then isolated NPCs from the DG of adult control, R26hGFAP, and R26Syn mice. Thus, the masking of X-gal staining difference in the adult DG (Figure 2A, left panels) was likely the result of the considerably reduced numbers of NPCs and immature neuron populations at this age compared with those of P10. Nonetheless, we demonstrate that in adult mice the Syn-Cre transgene can only elicit recombination in differentiated neurons, while the hGFAP-Cre transgene affects both NPCs and neurons.

Ablation of TrkB in Early Postnatal NPCs Impairs DG Morphogenesis

The conditional knockout animals, carrying either the hGFAP-Cre (TrkBhGFAP) or the Syn-Cre (TrkBSyn), are viable at birth and have normal survival rates (up to 15 months recorded) (Luikart et al., 2005). The TrkBhGFAP mice displayed a significant reduction in the volume of the DG granular layer (Figures 3A and 3B) that first became measurable at P10. The volume reduction stabilized at around 30% after the initial postnatal weeks and persisted throughout adulthood. This abnormality was not caused by changes in cell density or cell size (Figure 3D; p > 0.2), but rather was a result of decrease in the number of granule neurons, evidenced by a significant reduction in the thickness of the DG granular layer (Figure 3C; n = 6 for each, F2,15 = 5.477, p = 0.0164).
The number and morphology of glial cells were not appreciably affected in the TrkB\textsuperscript{hGFAP} mice (Figure S3 and not shown). The decreases in DG size and granular layer thickness were only observed in TrkB\textsuperscript{hGFAP} mice. Red lines and circles highlight the length of the granular layer and the size of single cells, respectively. Scale bars, 1 mm (hippocampus) and 10 \(\mu\)m (DG).

(B) Quantitative analysis revealed that the reduction in the DG volume of TrkB\textsuperscript{hGFAP} mice first became measurable at P10 and persisted in adulthood, \(n > 7\) for each.

(C) At P15, TrkB\textsuperscript{hGFAP} mice, but not TrkB\textsuperscript{Syn} mice, had thinner granular layer, demonstrated by a decrease in neuronal number. Data is shown as percentage of control. \(n = 6\) for each. \(F_{2,15} = 5.477, p = 0.0164\). GL, granular layer.

(D) Comparative analysis of the size and density of DG granular neurons from control and TrkB\textsuperscript{hGFAP} mice. Note the absence of difference in either category.

(E) DAPI staining of hippocampus sections from control and BDNF\textsuperscript{hGFAP} mice at the age of 2 months. Note the decrease in DG size in the BDNF\textsuperscript{hGFAP} mice. Scale bar, 200 \(\mu\)m.

(F) Quantitative analysis revealed a 30\% reduction in DG volume in the BDNF\textsuperscript{hGFAP} mice at the age of 2 months. \(n = 8–10\) for each.

Results are mean + SEM. *\(p < 0.05\), **\(p < 0.01\).

Ablation of TrkB Impairs Proliferation and Neurogenesis

Given the apparent reduction in the number of granule neurons in TrkB\textsuperscript{hGFAP} mice, but not TrkB\textsuperscript{Syn} mice, we examined whether lack of TrkB in the NPCs affected the neurogenic capacity of the SGZ. Control and TrkB\textsuperscript{hGFAP} mutant animals were evaluated at P15, during a period in which the DG undergoes dynamic morphogenesis (Altman and Bayer, 1990). The number of NeuroD-positive, newly generated neurons was drastically reduced in the TrkB\textsuperscript{hGFAP} mice, whereas the number in TrkB\textsuperscript{Syn} mice was comparable with that of the control mice (Figure 4A). Thus, TrkB ablation in the NPCs, but not in differentiated neurons, impairs hippocampal neurogenesis.

Reduced neurogenesis in the absence of TrkB could be caused by increased cell death, decreased proliferation, or a combination of both processes. We first examined programmed cell death using TdT-mediated dUTP nick end labeling (TUNEL) assay and immunohistochemistry for activated caspase 3. At all time points examined, the number of apoptotic cells detected with either method was uniformly low and no measurable

The number and morphology of glial cells were not appreciably affected in the TrkB\textsuperscript{hGFAP} mice (Figure S3 and not shown). The reduction of volume was most prominently observed in the hippocampus and DG, and did not appear to be a secondary result of a broader developmental defect, because the TrkB\textsuperscript{hGFAP} mice had normal body and brain size at all ages examined (Figure S2).

We also measured the volumes of other anatomical regions, such as the striatum, in 2-month-old TrkB\textsuperscript{hGFAP} mice and found normal volume (23.62 ± 0.94 mm\(^3\) in controls, 23.32 ± 1.06 mm\(^3\) in TrkB\textsuperscript{hGFAP}, \(n = 6\) for each, \(p > 0.2\)). The TrkB\textsuperscript{Syn} animals, contrary to the TrkB\textsuperscript{hGFAP} mice, displayed normal development of the DG granular layer (Figures 3A–3C). These observations demonstrate that TrkB expression in the SGZ is required for the normal structural development of the DG. The phenotypic difference between the TrkB\textsuperscript{hGFAP} and TrkB\textsuperscript{Syn} mice suggests a cell-autonomous function of TrkB in the DG NPCs. We have previously reported that conditional deletion of BDNF using the hGFAP-Cre transgene (BDNF\textsuperscript{hGFAP}) resulted in 80\% reduction of BDNF protein level in the hippocampus (Monteggia et al., 2007); we note that these mice also displayed significantly reduced DG granular layer volume, indicating a ligand-receptor coincidence (Figures 3E and 3F, \(n = 8–10\) for each genotype, \(p < 0.005\)).

Ablation of TrkB in Postnatal NPCs Impaired DG Morphogenesis

(A) Representative images of Nissl-stained DG sections from control, TrkB\textsuperscript{hGFAP}, and TrkB\textsuperscript{Syn} mice at P15. The decreases in DG size and granular layer thickness were only observed in TrkB\textsuperscript{hGFAP} mice. Red lines and circles highlight the length of the granular layer and the size of single cells, respectively. Scale bars, 1 mm (hippocampus) and 10 \(\mu\)m (DG).

(B) Quantitative analysis revealed that the reduction in the DG volume of TrkB\textsuperscript{hGFAP} mice first became measurable at P10 and persisted in adulthood, \(n > 7\) for each.

(C) At P15, TrkB\textsuperscript{hGFAP} mice, but not TrkB\textsuperscript{Syn} mice, had thinner granular layer, demonstrated by a decrease in neuronal number. Data is shown as percentage of control. \(n = 6\) for each. \(F_{2,15} = 5.477, p = 0.0164\). GL, granular layer.

(D) Comparative analysis of the size and density of DG granular neurons from control and TrkB\textsuperscript{hGFAP} mice. Note the absence of difference in either category.

(E) DAPI staining of hippocampus sections from control and BDNF\textsuperscript{hGFAP} mice at the age of 2 months. Note the decrease in DG size in the BDNF\textsuperscript{hGFAP} mice. Scale bar, 200 \(\mu\)m.

(F) Quantitative analysis revealed a 30\% reduction in DG volume in the BDNF\textsuperscript{hGFAP} mice at the age of 2 months. \(n = 8–10\) for each.

Results are mean + SEM. *\(p < 0.05\), **\(p < 0.01\).
An increase was observed in the TrkBhGFAP mice, indicating that lack of TrkB did not appreciably affect survival in vivo (Figure S3; n = 6 for each, p > 0.2).

We next assessed proliferation by measuring the number of cells that incorporate the DNA synthesis marker 5-bromo-2’-deoxyuridine (BrdU). P15 mice were treated with pulses of BrdU.

Figure 4. Lack of TrkB in NPCs Impaired Neurogenesis and Proliferation In Vivo and In Vitro

(A) Representative confocal images of the DG immunostained for NeuroD (red). Note the reduction of NeuroD-positive cells, representing immature neurons, in TrkBhGFAP mice, but not TrkBShGFAP mice (P15). Scale bar, 100 μm.

(B and C) Proliferation in the DG was decreased in TrkBhGFAP mice, but not TrkBShGFAP mice, evidenced by a reduction in Ki67-positive cells (C) or BrdU-positive cells (B and C). Scale bar, 100 μm. n = 7–9 for each. F2,21 = 78.39, p < 0.0001 (BrdU); F2,21 = 51.64, p < 0.0001 (Ki67).

(D and E) Cell-cycle analysis using BrdU pulsing and coimmunostaining for BrdU (green) and Ki67 (red) showed an increase in the ratio of BrdU-labeled cells that have exited the cell cycle (BrdU+Ki67−) in the DG of TrkBhGFAP mice. Arrowheads indicate BrdU+Ki67− cells. Scale bar, 100 μm. Data represent the ratio of (BrdU+Ki67−)/(All BrdU-positive).

(F) Western blots of lysates from DG-derived neurospheres probed for phospho-Trk490, TrkB, and actin, with and without BDNF stimulation. Note the abundance of TrkB expression and the increase of phospho-Trk in the presence of BDNF.

(G) Cells from the DG of adult mice were plated at equal density and allowed to proliferate in the presence of EGF and bFGF. The frequency of neurosphere formation was lower in the TrkBhGFAP mice, indicating a decrease in NPC population.

(H) Addition of BDNF facilitated the growth of primary neurospheres derived from the DG of control mice, but not of the TrkBhGFAP mice. TrkBhGFAP neurospheres grown without BDNF were also smaller than control, indicating impaired proliferation. n = 4 for each. ANOVA revealed significant effects of BDNF (F1,12 = 6.994, p = 0.0214), genotype (F1,12 = 102.2, p < 0.0001), and an interaction between the two (F1,12 = 19.3, p = 0.0009). Results are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001. Error bars, mean ±SEM.
and sacrificed 24 hr after the first injection. Within the area en-
compassing the inner granular layer and SGZ, the number of
BrdU-positive cells in the TrkB<sup>GFAP</sup> mice displayed a 48% re-
duction compared with that of control mice (Figures 4B and
4C; n = 7–9 for each; F<sub>2,21</sub> = 78.39, p < 0.0001; p < 0.001 for post
hoc test of control to TrkB<sup>GFAP</sup> comparison). Similarly,
the number of cells positive for Ki67, an endogenous marker
for actively cycling cells, was decreased by 51% in the SGZ of
the TrkB<sup>GFAP</sup> mice (Figures 4B and 4C; n = 7–9 for each; F<sub>2,21</sub> = 51.64, p < 0.0001; p < 0.001 for post hoc test of control to
TrkB<sup>GFAP</sup> comparison). Again, the numbers of BrdU-
or Ki67-positive cells were unaffected in the DG of the TrkB<sup>Syn</sup>
 mice (Figures 4B and 4C; p > 0.2 for control to TrkB<sup>Syn</sup>
comparisons).

To further investigate the cellular abnormality that leads to
the significant decrease of proliferation in the TrkB<sup>GFAP</sup>
mice, we evaluated cell-cycle exit of BrdU-incorporating cells by examining
their expression of Ki67 after a 2 hr chase period, at which point
cells that were labeled with BrdU during the S phase but have sub-
sequently left the cycle would lose their Ki67 expression (BrdU+;
Ki67–), whereas the ones that remained in active cell cycle would
be double positive (BrdU+; Ki67+). In the TrkB<sup>GFAP</sup>
mice 23.1% ± 3.9 % of all BrdU-positive cells were Ki67-negative,
displaying a 208% increase in cell-cycle exit over the control mice, while
only 7.5% ± 1.3 % were Ki67-negative (Figures 4D and 4E; 1053
brdU-positive cells from five control and 772 from five TrkB<sup>GFAP</sup>
 mice, p < 0.05). Collectively, these observations demonstrate that TrkB
is required for normal precursor proliferation in the hippocampus.

**Activation of TrkB In Response to BDNF Facilitates
Proliferation In Vitro**

Our finding that the TrkB<sup>GFAP</sup> mice, but not the TrkB<sup>Syn</sup> mice,
displayed impaired hippocampal neurogenesis suggested a cell-autonomous requirement for TrkB in NPCs. To further ex-
mamine their intrinsic properties, we cultured NPCs from the DG in
serum-free conditions. In the presence of epidermal growth fac-
tor (EGF) and basic fibroblast growth factor (bFGF), DG NPCs
formed neurospheres, which expressed TrkB receptor that could
be activated by exogenous BDNF (Figure 4F). Although maint-
aining adult-DG-derived primary neurospheres in media contain-
ing BDNF (50 ng/ml) for 7–10 days did not increase the
frequency of primary neurosphere formation (not shown), there
was a significant increase in the size of the neurospheres, sug-
uggesting that activation of TrkB facilitates the expansion of neuro-
sphere-forming cells (Figure 4H; n = 4 for each group, p < 0.05 for
post hock test with or without BDNF comparison in control cells).
Primary neurospheres derived from the DG of adult TrkB<sup>GFAP</sup>
mice, when plated at equal density, displayed reduction in both
the number (Figure 4G; n = 4 for each group, p < 0.005)
and size (Figure 4H; p < 0.005 for post hoc test of control to
TrkB<sup>GFAP</sup> comparison without BDNF; F<sub>1,12</sub> = 102.2, p < 0.0001
for genotype; F<sub>1,12</sub> = 6.994, p = 0.0214 for BDNF). The ability of
primary neurospheres to form secondary neurospheres was also impaired in the TrkB<sup>GFAP</sup>
mice (secondary-to-primary neurosphere ratio: 0.322 ± 0.058 in TrkB<sup>GFAP</sup>
and 0.843 ± 0.096 in control, n = 4 for each, p < 0.005). In addition, deletion of TrkB
abolished neurosphere sensitivity to BDNF stimulation (Figure 4H, p > 0.2). Using an Annexin V labeling assay, we exam-
ined the percentage of apoptotic cells in the neurosphere cul-
tures and did not observe significant differences between control
and TrkB<sup>GFAP</sup> cells with or without BDNF treatment (n = 4 for
each; with 50 ng/ml BDNF: 14.95% ± 1.38% in controls,
16.25% ± 2.65% in TrkB<sup>GFAP</sup>; without BDNF: 14.46% ±
1.62% in controls, 17.69% ± 2.56% in TrkB<sup>GFAP</sup>; F<sub>1,8</sub> = 1.136,
p = 0.3177 for genotype; F<sub>1,8</sub> = 0.0498, p = 0.8290 for BDNF),
suggesting that the reduction in the size of neurospheres derived from
TrkB<sup>GFAP</sup> mice was not due to survival deficits. Thus,
BDNF facilitates proliferation by acting directly on NPCs, and the activation of TrkB is solely responsible for this effect.

**TrkB Is Required for Induced Proliferation
and Neurogenesis by ADs and Voluntary Exercise**

Reduction in hippocampal volume has been observed in animal
models of stress (Coe et al., 2003; Czeh et al., 2001), which
may be reversed or prevented by chronic AD treatment (Sheline
et al., 2003). Similarly, reduction of hippocampal volume has
been reported in some studies of human patients with major de-
pression (Bremner et al., 2000; MacQueen et al., 2003) and post-
traumatic stress disorder (Gilbertson et al., 2002; Karl et al., 2006;
Smith, 2005). Although the cellular mechanism is unclear in hu-
mans, animal studies have demonstrated that chronic exposure
to various types of ADs induces DG proliferation and neurogene-
sis (Malberg et al., 2000), thereby potentially contributing to the
recovery of volume loss. In this context, we examined whether
chronic AD treatment can restore neurogenesis in TrkB<sup>GFAP</sup>
mice. We thus treated control, TrkB<sup>GFAP</sup>, and TrkB<sup>Syn</sup> mice with daily injections of the serotonin reuptake inhibitor fluoxetine
(10 µg/g) or the tricyclic imipramine (20 µg/g) for 21 days (n = 7–29
for each group; see Table S1 available online). As previously es-

d, in the control mice, both drugs increased the number of
Ki67-positive cells in the DG compared to saline (Figures 5A and
5B; F<sub>2,98</sub> = 40.48, p < 0.0001 for treatment; p < 0.001 for post hoc
test of both AD-to-saline comparisons). The induction of prolifer-
ation was echoed by an increase in the number of newly gener-
ated neurons expressing Doublecortin and NeuroD (Figures 5C
and 5D and not shown; F<sub>2,98</sub> = 30.79, p < 0.0001 for treatment;
< 0.001 for post hock test of both AD-to-saline comparisons).
In contrast, TrkB<sup>GFAP</sup> mice treated with the same ADs did not
show an increase in the number of proliferating cells or immature
neurons (Figures 5A–5D; F<sub>2,98</sub> = 332.3, p < 0.0001 for the effect of

genotype on Ki67; F<sub>2,98</sub> = 211.6, p < 0.0001 on Doublecortin). This
induction, or an extended 6 week treatment, also failed to restore
the DG volume deficit in the TrkB<sup>GFAP</sup> mice (not shown). The
TrkB<sup>Syn</sup> mice responded normally to both ADs (Figures 5A–5D;
< 0.001 for post hoc test of both AD-to-saline comparisons).

In rodents, voluntary exercise such as wheel-running behavior has
been demonstrated to robustly induce neurogenesis, much in the
same fashion as AD treatment (van Praag et al., 1999). Less
is known, however, about the underlying mechanism of this
AD-like effect of exercise. To determine whether TrkB is also re-
quired for this process, we subjected control and TrkB<sup>GFAP</sup>
mice to 6 weeks of wheel-running (n = 8–16 for each group; Table
S2). Both the numbers of Ki67-positive and Doublecortin-positive
cells increased in control runners, compared with control
sedentary animals (Figure 6C; F<sub>1,136</sub> = 13.64, p = 0.0007 for exer-
cise; Figure 6D: F<sub>1,38</sub> = 16.01, p = 0.0033; p < 0.001 for post hoc
test of both comparisons). A significant increase of BDNF protein

---

level was also observed in the hippocampus of runners (66.44 ± 3.65 pg/mg in runners; 45.83 ± 2.99 pg/mg in sedentary controls; n = 3 for each; p < 0.05). The TrkB<sup>GFAP</sup> mice, despite normal running distance (5.96 ± 0.41 km/day in controls, 5.48 ± 0.34 km/day in TrkB<sup>GFAP</sup>, p > 0.2) as well as elevation of BDNF level in runners (60.33 ± 4.43 pg/mg in runners; 40.94 ± 1.37 pg/ml in sedentary controls; n = 3 for each; p < 0.05), did not show any increase in proliferation and neurogenesis (Figures 6C and 6D, p > 0.2 for both). Since we tested male and female mice in the voluntary exercise paradigm, we further separated the results into gender- and genotype-specific groups and found no statistically significant difference between males and females of the same genotype (not shown).

**TrkB Is Required for Behavioral Improvement Induced by ADs and Exercise**

To determine whether the lack of neurogenic response in the TrkB<sup>GFAP</sup> mice was coupled with general insensitivity to chronic ADs and exercise, we compared the depression- and anxiety-like behaviors in control and TrkB<sup>GFAP</sup> mice. First, mice treated with fluoxetine, imipramine, or saline for 21 days were examined in the novelty-suppressed feeding test (NSFT), a conflict paradigm in which the latency to feed in a novel environment is used as an indicator of anxiety level (Santarelli et al., 2003). In agreement with the general capacity of chronic ADs to ease anxiety, 24 hr after the last dose, control mice receiving fluoxetine or imipramine displayed significantly shorter latency to feed compared with saline-treated control mice (Figure 6A: F<sub>2,100</sub> = 8.022, p = 0.0006 for treatment; p < 0.001 for post hoc test for both AD-to-saline comparisons). The TrkB<sup>GFAP</sup> mice, on the contrary, were insensitive to the effects of either AD (Figure 6A: F<sub>2,100</sub> = 10.49, p < 0.0001 for genotype). Similarly, TrkB<sup>GFAP</sup> mice exposed to 6 weeks of running showed no improvement in the NSFT, whereas the control runners displayed clear decrease in latency compared with sedentary animals (Figure 6E: F<sub>1,41</sub> = 15.09, p = 0.0004 for genotype; p < 0.05 for post hoc test of running effect in control mice). No difference in home cage consumption or body weight loss was observed across genotypes (not shown).
Next we examined depression-like behavior in the control and TrkB<sub>hGFAP</sub> mice by using the tail-suspension test (TST), a paradigm of inescapable stress (Porsolt et al., 1987). All mice were tested 48 hr after the last dose of AD or saline to exclude the acute effects of AD on behaviors that do not correlate with prior duration of drug treatment, or clinical responses. Control runners and AD-treated control mice showed decreased immobility (immobility being a state of “behavioral despair”) compared with sedentary or saline-treated control mice, respectively (Figure 6F: \( F_{1,41} = 9.082, p = 0.0044 \) for genotype; Figure 6E: \( F_{2,100} = 8.022, p = 0.0008 \) for genotype and AD treatment). The TrkB<sub>hGFAP</sub> mice again failed to display any appreciable response to either treatment (Figure 6B: \( F_{2,100} = 4.233, p = 0.0172 \) for genotype; Figure 6F: \( F_{1,41} = 9.082, p = 0.0044 \) for genotype).

Despite the lack of response to ADs and exercise in the behavioral paradigms of NSFT and TST, the saline-treated TrkB<sub>hGFAP</sub> mice performed similarly compared to the control mice, suggesting relatively normal depression- and anxiety-like behaviors at the basal level. To further investigate this finding, we examined a cohort of control and TrkB<sub>hGFAP</sub> mice in a series of behavioral measures (Figure S5; \( n = 17–21 \) for each). We observed no significant differences within these two groups in the dark-light test in either the length of time spent or activity in the light compartment. In the open field test, the TrkB<sub>hGFAP</sub> mice were equivalent to controls in the time spent in the center. In the elevated-plus maze test, the TrkB<sub>hGFAP</sub> mice spent more time in the open arm compared with littermate controls. Together these results suggest normal (and in some cases reduced) baseline anxiety-like behaviors in the TrkB<sub>hGFAP</sub> mice. Similarly, in the forced swim test, the TrkB<sub>hGFAP</sub> mice displayed normal latency to immobility, and a nonsignificant trend toward decreased total length of immobility, thereby supporting earlier observations of normal baseline depression-like behavior. Based on the above observations, we conclude that the lack of response to ADs and exercise in the TrkB<sub>hGFAP</sub> mice cannot be explained by alterations in baseline behavior, but rather is a result of insensitivity to the molecular and cellular changes induced by chronic ADs and exercise.

### Normal Sensitivity to Chronic ADs in Mice Lacking TrkB in Differentiated Neurons

To explore whether the deficit in increased neurogenesis contributed to the abolished behavior sensitivity to chronic ADs,
we tested the TrkB<sup>Syn</sup> mice in the TST and the NSFT. Similar to control mice, upon chronic treatment with fluoxetine and imipramine, the TrkB<sup>Syn</sup> mice showed significant decrease in anxiety (Figure 6A; post hoc p < 0.05 for both ADs) and depression-like behaviors (Figure 6B; post hoc p < 0.05 for both ADs). This result, in conjunction with the observation that chronic AD treatment increased DG proliferation and neurogenesis in these mice (Figure 5), indicates that despite the lack of TrkB in differentiated neurons, the unaffected TrkB signaling in the NPCs was sufficient for the TrkB<sup>Syn</sup> mice to display a behavioral response to chronic ADs.

**Specific Ablation of TrkB in Adult NPCs Is Sufficient to Block Sensitivity to ADs**

To further delineate whether TrkB function in adult NPCs alone was required for AD-induced neurogenic and behavioral responses, we utilized a tamoxifen-inducible form of Cre recombinase, Cre<sub>ER<sup>2</sup></sub>, expressed under the regulatory element of the Nestin gene, allowing for TrkB ablation specifically in the adult neurogenic niches. When the Nestin-Cre<sub>ER<sup>2</sup></sub> mice were interbred with the R26 reporter mice (R26<sup>Nestin</sup>), Cre activity could be visualized either in embryonic or in adult CNS in a tamoxifen-dependent manner. Specifically, when 1-month-old R26<sup>Nestin</sup> mice were injected with vehicle or tamoxifen and analyzed 1 month afterward, spontaneous recombination (i.e., in vehicle-treated mice) was minimal, while tamoxifen-induced recombination was restricted to the DG, subventricular zone (SVZ), rostral migratory stream, olfactory bulb, and cerebellum (Figure 7A and not shown). In the DG, recombination occurred specifically in the inner granular layer and SGZ. To evaluate the efficiency of Nestin-Cre<sub>ER<sup>2</sup></sub> in targeting NPCs, we induced R26<sup>Nestin</sup> mice at 1 month and analyzed them at 2 and 7 months of age. The number of X-gal-stained cells was dramatically increased in the 6 months postinjection group, indicating effective recombination in the NPCs that were capable of proliferation and self-renewal (Figure 7B). Additional confirmation was obtained by double-immunostaining for β-gal and Doublecortin at 4 months of age, where the majority of Doublecortin-positive cells also coexpress β-gal (Figure 7C).

As described above, hGFAP-Cre-mediated ablation of TrkB resulted in a smaller DG that we attribute to the lack of TrkB signaling in early postnatal NPCs, which sustains rapid proliferation required for normal structural development. To bypass this essential phase of DG postnatal morphogenesis, we subjected TrkB<sub>lox/lox</sub>,Nestin-CreER<sub>2</sub> mice to tamoxifen (TrkB<sub>Nestin</sub>) at 1 month of age. Littermate TrkB<sub>lox/lox</sub>,Nestin-CreER<sub>2</sub> mice injected with vehicle, and TrkB<sub>lox/lox</sub> mice injected with tamoxifen, were analyzed and collectively presented as the control group. At the age of 6 weeks and 3 months, TrkB mRNA was virtually undetectable by RT-PCR in the DG NPCs of TrkB<sub>Nestin</sub> mice using FACSt-sorted Nestin-GFP-positive cells (Figure 7D). TrkB protein was also absent from the proliferating cells of the SGZ, while its level in the majority of granular neurons was unaffected (Figure 7E). As expected, ablation of TrkB at 1 month did not lead to a reduction in DG granular layer volume when examined at 3 months of age (Figure 7F; n = 6 for each; p > 0.2). Basal proliferation in the SGZ at this age (as measured by Ki67 staining) was modestly decreased in the TrkB<sub>Nestin</sub> mice, whereas no aberrant cell death was observed (Figure 5). Upon exposure to fluoxetine (10 µg/g) or imipramine (20 µg/g) for 21 days (n = 8–10 for each), the TrkB<sub>Nestin</sub> mice did not display increased NPC proliferation as measured by the numbers of Ki67-positive cells (Figure 7G: F<sub>1,50</sub> = 176.7, p < 0.0001 for genotype; F<sub>2,50</sub> = 12.45, p < 0.0001 for treatment; F<sub>2,50</sub> = 13.57, p < 0.0001 for interaction) and phosphorylated-histone-H3-positive cells (Figure S6; F<sub>1,50</sub> = 131.9, p < 0.0001 for genotype; F<sub>2,50</sub> = 17.46, p < 0.0001 for treatment; F<sub>2,50</sub> = 17.34, p < 0.0001 for interaction). The number of newly generated neurons as labeled by Doublecortin also did not change in the TrkB<sub>Nestin</sub> mice compared to the control group (Figure 7H: F<sub>1,50</sub> = 141.9, p < 0.0001 for genotype; F<sub>2,50</sub> = 7.014, p = 0.0021 for treatment; F<sub>2,50</sub> = 12.56, p < 0.0001 for interaction). The absence of neurogenic response in the TrkB<sub>Nestin</sub> mice coincided with a lack of behavioral improvements in both the NSFT (Figure 7I: F<sub>1,50</sub> = 13.68, p = 0.0005 for genotype) and the TST (Figure 7J: F<sub>1,50</sub> = 18.15, p < 0.0001 for genotype). These observations refine and confirm the preceding studies, indicating that ablation of TrkB from adult NPCs alone is sufficient to block sensitivity to chronic ADs.

**DISCUSSION**

**Regulation of Postnatal and Adult Neurogenesis in the DG**

The identification of NPCs in the largely postmitotic adult brain has transformed our perspective on the development, physiology, and pathology of this organ. Anatomically, NPCs in the postnatal and adult brain reside in the SVZ of the lateral ventricle and the SGZ of the DG, regions considered to be residues of the embryonic germinal niche (Alvarez-Buylla and Lim, 2004). However, adult NPCs, especially those in the DG, deviate from "embryonic properties" and have been shown to possess limited self-renewal, proliferation, and migration capacity (Bull and Bartlett, 2005; Seaberg and van der Kooy, 2002). Paradoxically, DG NPCs gain a remarkable capacity to respond to various extrinsic and intrinsic stimuli (Lledo et al., 2006; Ming and Song, 2005). The signaling mechanisms that specify these unique properties of DG NPCs, both on the basal level and the induced state, remain unidentified.

Our study shows that TrkB-expressing NPCs in the postnatal and adult DG respond to BDNF. As demonstrated in the TrkB<sub> GFP</sub> mice, ablation of TrkB from the DG NPCs blocked BDNF-induced neurosphere growth in vitro, and impaired proliferation and neurogenesis in vivo. Although the Cre-mediated deletion of TrkB in the TrkB<sub> GFP</sub> mice was not restricted to DG NPCs, the requirement for TrkB in proliferation was cell autonomous as evidenced by the facts that (1) neurospheres generated from the DG of the TrkB<sub> GFP</sub> mice had impaired proliferation in vitro; (2) in vivo, the dividing cells in the DG of the TrkB<sub> GFP</sub> mice were more prone to prematurely exit the cell cycle; (3) proliferation in the DG was unaffected in the TrkB<sub>Syn</sub> mice where Cre-mediated recombination occurred exclusively in differentiated neurons; and (4) temporal- and spatial-specific ablation of TrkB in young adult NPCs (of TrkB<sub>Nestin</sub>) also resulted in impaired proliferation and neurogenesis.

Neurotrophic factors, particularly BDNF, have been shown to promote proliferation of NPCs in vitro (Barnabe-Heider and Miller, 2003). Whether this effect resonates with a similar physiological
requirement in vivo is less apparent. BDNF germline heterozygous mice have been reported to have decreased (Lee et al., 2000) or increased (Sairanen et al., 2005) proliferation, as well as decreased (Sairanen et al., 2005) or normal (Rossi et al., 2006) long-term neurogenesis. By directly ablating the BDNF receptor, TrkB, in NPCs, our data ascertain an unambiguous, cell-autonomous requirement for TrkB in maintaining proliferation and neurogenesis in the DG. It is worth noting that because TrkB is the high-affinity receptor for both BDNF and neurotrophin 4 (NT4), the phenotype observed in the TrkB*GFP* mice could be a combinatorial outcome of a lack of signaling response to both ligands, although the expression level of NT4 in the postnatal brain is lower than that of BDNF. We have examined the BDNF*GFP* conditional mutant mice and observed a degree of

![Figure 7. Specific Ablation of TrkB in Adult NPCs Was Sufficient to Block AD Sensitivity](image-url)

(A) X-gal staining on sagittal brain sections of R26*Nestin* mice treated with vehicle or tamoxifen at 1 month and analyzed at 2 months of age.  
(B) X-gal staining on DG sections of R26*Nestin* mice treated with tamoxifen at 1 month and analyzed 1 month or 6 months afterwards. Note the increase in X-gal-stained cells 6 months after tamoxifen injection. Scale bar, 200 μm.  
(C) Costaining for β-gal (green) and Doublecortin (red) on DG sections from R26*Nestin* mice treated with tamoxifen at 1 month and analyzed at 4 months of age. Scale bar, 10 μm.  
(D) RT-PCR detection of TrkB and G3PDH transcripts in FACS-sorted Nestin-GFP-positive and -negative cells from the DG of TrkB*flx/flx*:Nestin-CreERT2 mice treated with tamoxifen (TrkB*Nestin*) or vehicle (control) at 1 month old.  
(E) Costaining for TrkB (green) and Ki67 (red) on DG sections from TrkB*Nestin* mice at the age of 3 months (2 months posttamoxifen injection). Note the lack of colocalization of TrkB and Ki67 in the SGZ. Scale bars, 10 μm and 5 μm.  
(F) TrkB*Nestin* mice at the age of 3 months had normal DG volume.

(G and H) Quantitative analysis revealed no increase in the number of Ki67-positive (G) or Doublecortin-positive (H) cells in the TrkB*Nestin* mice after fluoxetine or imipramine treatments. n = 8–10 for each. ANOVA (GLM) found significant effects of AD treatment (F2,50 = 12.45, p < 0.0001 for Ki67; F2,50 = 7.014, p = 0.0021 for Doublecortin), genotype (F1,50 = 176.7, p < 0.0001 for Ki67; F1,50 = 141.9, p < 0.0001 for Doublecortin), and an interaction of the two (F2,50 = 13.57, p < 0.0001 for Ki67; F2,50 = 12.56, p < 0.0001 for Doublecortin).

(I and J) TrkB*Nestin* mice did not display decrease in latency to feed (I) or duration of immobility (J) after chronic exposure to fluoxetine or imipramine, compared to control mice. Data are shown as percentage of control treated with saline. n = 8–10 for each. NSFT: F1,50 = 13.68, p = 0.0005 for genotype; F2,50 = 4.206, p = 0.0206 for the interaction of genotype and AD treatment. TST: F1,50 = 18.15, p < 0.0001 for genotype; F2,50 = 6.848, p = 0.0024 for AD and F2,50 = 9.488, p = 0.0003 for an interaction of the two.

Results are mean ± SEM. *p < 0.05, ***p < 0.001.
Neuron
TrkB Governs Sensitivity to Antidepressants

reduction in DG granular layer volume similar to that of the TrkB<sup>GFP</sup> mice. NT4 null mice are viable (Liebl et al., 2000) and appear to have normal DG volume (not shown).

The specific impairment of postnatal DG morphogenesis observed in the TrkB<sup>GFP</sup> mice appears to be a unique phenomenon. Although NPCs in the embryonic CNS express TrkB and have been shown to benefit from exogenous BDNF in vitro (Bar-nabe-Heider and Miller, 2003), ablation of TrkB or BDNF in vivo, as demonstrated in TrkB and BDNF germine knockouts as well as the TrkB<sup>GFP</sup> mice, does not result in aberrant neurogenesis of the prenatal CNS. The TrkB<sup>GFP</sup> mice were born with, and continued to have, normal brain size throughout adulthood. The reduction of the DG granular layer volume was absent at birth, and only becomes appreciable after P10. Absence of TrkB did not affect proliferation in the primary dentate neuroepithelium at E16.5, or the migration and resettling of the secondary dentate matrix at P0 (not shown). Therefore, we reveal here a regulatory mechanism that uncouples postnatal DG neurogenesis from embryonic development. Interestingly, while early ablation of TrkB (TrkB<sup>GFP</sup>) dramatically reduced proliferation in adult DG, we observed a more modest impairment in basal proliferation when TrkB is removed at the young adult age (TrkB<sup>Nestin</sup>). This likely reflects the combined effects of TrkB ablation in the early postnatal period on reducing individual cell division (by promoting cell cycle exiting) and diminishing the overall pool of progenitor cells available for proliferation. In contrast, late deletion of TrkB cannot significantly disturb the progenitor pool size, and thus the phenotype in the TrkB<sup>Nestin</sup> mice likely represents the role of TrkB in regulating the behaviors of individual adult NPCs.

Our studies demonstrate that deletion of TrkB from DG NPCs (TrkB<sup>GFP</sup> and TrkB<sup>Nestin</sup>) abolishes the proliferative and neurogenic effects of chronic AD treatments and voluntary exercise. This is consistent with earlier observations that both ADs and running induce significant increases in BDNF level (Neuper et al., 1996; Nibuya et al., 1995). The inability of TrkB null NPCs to respond to ADs and running was most likely due to the lack of sensitivity to BDNF, and not from developmental deficits that compromise cell division, as evidenced by their capacity to react to other exogenous factors including EGF, bFGF, and oxygen conditions in vitro (not shown). Conversely, when TrkB was ablated only from differentiated neurons (TrkB<sup>Syn</sup>), both the proliferation and neurogenesis responses to chronic ADs were maintained. It is intriguing that we observed a slightly more robust response to ADs in the TrkB<sup>Syn</sup> mice, suggesting that when surrounded by TrkB null neurons, NPCs and immature neurons with unaffected TrkB signaling may have a selective advantage in growth, survival, or both.

TrkB and the Behavioral Efficacy of ADs
There has been considerable evidence that genetic ablation of BDNF or TrkB may interfere with the normal function of the adult brain, depending on the regions affected. Conditional knockout animals lacking TrkB in forebrain neurons (with broader recombination than TrkB<sup>GFP</sup> and TrkB<sup>Syn</sup>) have impaired spatial learning behavior, but display normal anxiety level in the open field test (Minichiello et al., 1999). The latter is consistent with our observation that the TrkB<sup>GFP</sup> mice exhibited normal basal anxiety-like behavior in the NSFT, the open field test, and the dark-light preference test. Likewise, the TrkB<sup>GFP</sup> mice seemed to have normal depression-like behavior at the basal level, as measured by the TST and the forced swim test. The TrkB<sup>Nestin</sup> mice only lack TrkB in a highly specific population of cells, and were phenotypically indistinguishable from control mice at baseline. Previous studies with BDNF mutants regarding these anxiety- and depression-like behaviors have yielded mixed results: some were normal (Gorski et al., 2003; Monteggia et al., 2004; Saarelainen et al., 2003) while others were not (Chen et al., 2006; Monteggia et al., 2007; Rios et al., 2001). This discrepancy may be explained by the differences in the brain regions affected by the genetic ablation. BDNF is a secreted protein that can be transported and deposited across long distances in both retrograde and anterograde directions. Therefore, local inactivation of the BDNF gene may well result in global disturbance of BDNF protein level, thus confounding the interpretation of results. The recent discovery that BDNF in different regions of the brain may have opposing functions in modulating the stress response supports this notion (Berton et al., 2006).

Unlike control mice, the TrkB<sup>GFP</sup> (and TrkB<sup>Nestin</sup>) mice were insensitive to chronic ADs and exercise-induced improvement in depression- and anxiety-like behaviors. This finding provides compelling evidence that TrkB is required for a shared molecular mechanism through which ADs and exercise act. This process is likely to be BDNF dependent, echoing previous studies showing elevated levels of BDNF with chronic ADs (Nibuya et al., 1995) as well as blunted behavioral response to ADs in BDNF mutant animals (Chen et al., 2006; Monteggia et al., 2004; Saarelainen et al., 2003). Interestingly, TrkB<sup>Syn</sup> mice show normal behavioral responses to chronic AD treatment, indicating that neuronal expression of TrkB is not required for the behavioral efficacy of ADs. Though the mechanism via which ADs elicit increase in hippocampal BDNF level remains undefined, this process only occurs after chronic exposure to ADs (Nibuya et al., 1995), implicating BDNF elevation and the subsequent TrkB activation as an indirect downstream event from the acute accumulation of monoamine, such as serotonin and noradrenaline. It has been suggested that chronic ADs promote the cAMP pathways and increase CREB activity (Nibuya et al., 1996)—CREB being a transcription factor that activates the BDNF gene, among a multitude of other targets. While these observations provide important insight into the molecular underpinning of AD response, future work is needed to validate and further delineate the involvement of this pathway in regulating neurogenesis and mood (Conti et al., 2002; Nakagawa et al., 2002).

Functional Link between Neurogenesis and Sensitivity to ADs
It has been debated whether neurogenesis in the adult hippocampus bears functional significance in the action of chronic ADs, or the physiology of the hippocampus in general (Leuner et al., 2006; Scharfman and Hen, 2007; Zhao et al., 2008). In the current study, we selectively abolished the neurogenic sensitivity and the corresponding behavioral responses to chronic ADs by ablating TrkB in adult NPCs (TrkB<sup>Nestin</sup>) and NPCs and neurons (TrkB<sup>GFP</sup>), but not in neurons alone (TrkB<sup>Syn</sup>). Therefore, we conclude that a TrkB-dependent increase in neurogenesis is required for the effects of chronic ADs in control and
TrkB<sup>Syn</sup> mice in behavioral paradigms of NFST and TST. The hGFAP-Cre and Nestin-Cre<sup>ERT2</sup> transgenes elicit recombination in the neurogenic niches of SVZ and hippocampus. However, prior studies have excluded a possible link between SVZ neurogenesis and the behavioral effects of ADs in rodents (Malberg et al., 2000; Santarelli et al., 2003). Thus, our data provide genetic and mechanistic insight to previous reports that irradiation-mediated ablation of dividing cells from the hippocampal region of rodents abolished their behavioral responses to ADs (Airan et al., 2007; Santarelli et al., 2003; Wang et al., 2008a). A recent study reports differential response to chronic ADs in inbred strains of mice, suggesting differing molecular and cellular mechanisms (Holick et al., 2008). Thus, in contrast to the present findings and previous reports (Encinas et al., 2006; Santarelli et al., 2003; Wang et al., 2008a), chronic fluoxetine produces antidepressive behavioral effects in BALB/cJ mice by mechanisms independent of serotonin 1A receptor and DG neurogenesis. This marked strain difference highlights the existence of multiple mechanisms by which chronic ADs change anxiety- and depression-like behaviors, and raises the intriguing possibility that genetic variations may be involved in determining the path of AD efficacy.

Our results also suggest that reduced levels of basal proliferation in the DG of TrkB<sup>flxb/flox</sup> and TrkB<sup>Nestin</sup> mice do not directly lead to obvious affective impairment, an observation consistent with recent findings using an irradiation-mediated NPC ablation method (Airan et al., 2007; Santarelli et al., 2003; Wang et al., 2008a). Rather, these animal studies suggest ADs and exercise-stimulated increase in DG neurogenesis may translate into enhanced synaptic plasticity in the pertinent neural circuits (Wang et al., 2008a), which subsequently manifests as behavioral responsiveness to such treatments. One possible explanation for this dissociation between basal and induced neurogenesis may reside in functional differences between neurons generated at the constant state and stimulated state (Jakubs et al., 2006). The exact nature of these differences and the molecular mechanism that encodes them remain to be determined. Nonetheless, our data suggest that TrkB regulates DG NPCs in both states.

### EXPERIMENTAL PROCEDURES

A detailed description of all procedures is included in the Supplemental Data.

### Animals

The flox alleles of TrkB and BDNF were generated previously (Luikart et al., 2005; Monteggia et al., 2007). All mouse protocols were approved by the Institutional Animal Care and Research Advisory Committee at University of Texas Southwestern Medical Center.

### Histology and Quantitative Analysis

Mice were intracardially perfused with PBS followed by 4% (w/v) paraformaldehyde (PFA) in PBS, and the dissected brains were postfixed in 4% PFA at 4°C. In situ hybridization, TUNEL assay, X-gal, Nissl, and immunostaining were performed as previously described (Lei et al., 2005; Luikart et al., 2005). Detailed methods for these assays and the stereological quantifications are described in Supplemental Experimental Procedures.

### Protein Analysis

Total protein was extracted and measured for western blotting and BDNF ELISA assay. Total protein from the supernatant was used for western blotting as described (Lush et al., 2008). BDNF level was measured using a BDNF ELISA kit (Promega) as per manufacturer’s instructions.

### Neurosphere Cultures

Neurosphere culture was established and maintained as described (Bull and Bartlett, 2005) with some modifications. Briefly, DG of adult mice were dissected (Seaberg and van der Kooy, 2002) and digested in 0.1% trypsin-EDTA (Sigma) followed by mechanical trituration until smooth. Cells were plated at a density of 20 cells/μl in complete growth medium consisting of mouse NeuroCult NSC basal medium (StemCell Technologies), mouse NeuroCult NSC proliferation supplements (StemCell Technologies), 2 μg/ml heparin (Sigma), EGF (20 ng/ml, GibCO), and bFGF (10 ng/ml, Sigma). Treatment with BDNF (60 ng/ml, R&D Systems) started from the first day, or 30 min before harvest in the case of phospho-Trk<sub>G90</sub> western blotting.

### FACS

For FACS isolation of GFP-positive or -negative cells, primary cells were prepared from the DG of Nestin-GFP transgenic mice at the ages of P2, P15, and 2 months by following the procedure for establishing neurosphere culture. GFP-positive and -negative fractions of the live cells (propidium iodide negative cells) were analyzed and sorted on FACS Aria.

### Tamoxifen Treatment

Tamoxifen (Sigma) was dissolved in a sunflower oil/ethanol mixture (9:1) at 6.7 mg/ml. Vehicle (9:1 sunflower oil/ethanol) or tamoxifen was injected intraperitoneally to 1-month-old mice at 250 μg/20 g (0.83 mg/kg) twice a day for 5 consecutive days. All injected mice were observed daily for neurological-related abnormality. TrkB<sup>flxb/flox</sup>;Nestin-Cre<sup>ERT2</sup> mice injected with vehicle and TrkB<sup>flxb/flox</sup>;Nestin-Cre<sup>ERT2</sup> mice injected with tamoxifen were indistinguishable and therefore pooled as the control group.

### AD Treatment, Voluntary Exercise, and Behavioral Tests

All animals used for AD treatment, running, and subsequent behavioral tests were littermates. Detailed information on the cohorts of animals used and the procedures of behavioral tests are described in Supplemental Data. Briefly, immediately after the last dose of AD or saline, or the last day of running, mice were deprived of food for 24 hr and then subjected to the NSFT. Twenty-four hours afterwards, the same groups of mice were subjected to the TST. Latency to feed in the NSFT and length of immobility in the TST were rated as previously described (Santarelli et al., 2003; Svenningsson et al., 2006), with investigators blind to genotypes. Mice were sacrificed 24 hr after the TST for tissue collection and subsequent analyses.

### SUPPLEMENTAL DATA

The Supplemental Data for this article include Supplemental Experimental Procedures, two tables, and six figures and can be found online at http://www.neuron.org/cgi/content/full/59/3/399/DC1/.

### ACKNOWLEDGMENTS

The authors would like to thank Shawn Kennedy, Dustin Corgan, Linda McClellan, Steven McKinnon for technical assistance, Drs. Jingsheng Yan and Joshua Koch for advice on statistical analyses, and Dr. Louis Reichardt for providing the TrkB antibody. We are indebted to the members of the Parada lab, in particular Drs. Lei Lei and Mark Lush for sharing reagents and helpful suggestions. We wish to thank Dr. Eric J. Nestler for insightful discussions and Dr. Amelia Eisch for critical reading of this manuscript. This work was supported by the NINDS grant R37NS033199, NIMH Conte Center grant P50MH66172, and the ACS.

Accepted: June 20, 2008
Published: August 13, 2008

REFERENCES


Neuron
TrkB Governs Sensitivity to Antidepressants

Cell Press

Neuron 59, 399–412, August 14, 2008 ©2008 Elsevier Inc. 411


