Behavioral and serotonergic consequences of decreasing or increasing hippocampus brain-derived neurotrophic factor protein levels in mice

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Abstract

Antidepressants such as Selective Serotonin Reuptake Inhibitors (SSRI) act as indirect agonists of serotonin (5-HT) receptors. Although these drugs produce a rapid blockade of serotonin transporters (SERTs) in vitro, several weeks of treatment are necessary to observe clinical benefits. This paradox has not been solved yet. Recent studies have identified modifications of intracellular signaling proteins and target genes that could contribute to antidepressant-like activity of SSRI (e.g., increases in neurogenesis and BDNF protein levels), and may explain, at least in part, their long delay of action. Although these data suggest a positive regulation of 5-HT on the expression of the gene coding for BDNF, the reciprocal effects of BDNF on brain 5-HT neurotransmission remains poorly documented. To study the impact of BDNF on serotonergic activity, a dual experimental strategy was used to analyze neurochemical and behavioral consequences of its decrease (strategy 1) or increase (strategy 2) in the brain of adult male mice. (1) In heterozygous BDNF−/+ mice in which brain BDNF protein levels were decreased by half, an enhancement of basal extracellular 5-HT levels (5-Htext) that induced a down-regulation of SERT, i.e., a decrease in its capacity to reuptake 5-HT, was found in the hippocampus. In addition, the SSRI, paroxetine, failed to increase hippocampal 5-Htext in BDNF−/+ mice, while it produces robust effects in wild-type littermates. Thus, BDNF−/+ mice can be viewed as an animal model of genetic resistance to serotonergic antidepressant drugs. (2) In wild-type BDNF+/+ mice, the effects of intra-hippocampal (vHi) injection of BDNF (100 ng) in combination with a SSRI was examined by using intracerebral microdialysis and behavioral paradigms that predict an antidepressant- and anxiolytic-like activity of a molecule [the forced swim test (FST) and the open field paradigm (OF) respectively]. BDNF induced a rapid and transient increase in paroxetine response on 5-Htext in the adult hippocampus, which was correlated with a potentiation of its antidepressant-like activity in the FST. The effects of BDNF were selectively blocked by K252a, an antagonist of its high-affinity TrkB receptor. Such a correlation between neurochemical and behavioral effects of BDNF + SSRI co-administration suggests that its antidepressant-like activity is linked to the activation of 5-HT neurotransmission in the adult hippocampus. BDNF also had a facilitatory effect on anxiety-like behavior in the OF test, and paroxetine prevented this anxiogenesis. What was the mechanism by which BDNF exerted these latter effects? Surprisingly, by using zero net flux method of quantitative microdialysis in vivo, we found that an intra-hippocampal BDNF injection in wild-type mice decreased the functional activity of SERT as observed in BDNF−/+ mice. However, the decreased capacity of SERT to reuptake 5-HT was not associated to an increase in basal 5-Htext in the hippocampus of WT mice. Interestingly, using in situ hybridization experiments indicated that TrkB receptor mRNA was expressed in the hippocampus and dorsal raphe nucleus in adult mice suggesting that the neurochemical and behavioral effects of intra-hippocampal BDNF injection can mobilize both pre- and post-synaptic elements of the brain 5-HT neurotransmission. Taken together, these set of experiments unveiled a relative opposition of neurochemical and behavioral responses following either a decrease (in BDNF+/− mutant mice) or an increase in brain BDNF levels (bilateral intra-hippocampal injection) in adult mice. In view of developing new antidepressant drug strategy, a poly-therapy combining BDNF with a chronic SSRI treatment could thus improve the efficacy of current medications.

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1. Introduction

Antidepressants such as Selective Serotonin Reuptake Inhibitors (SSRI) act as indirect agonists of monoamine receptors. Although SSRI drugs produce relatively rapid blockade of serotonin (5-HT) transporters (SERT) in vitro, the onset of clinical benefits usually takes several (4–6) weeks to occur. The paradox between SSRI near-immediate effects on brain neurotransmitter systems and the slow symptomatic recovery in treating major depressive episodes has not been solved yet. Recent years have provided new information about changes in neurogenesis (mainly cell proliferation) and brain-derived neurotrophic factor (BDNF) protein levels in the adult hippocampus following chronic treatment with antidepressant drugs. Indeed, chronic, but not acute, SSRI treatment by increasing 5-HT neurotransmission causes an increase in BDNF expression (mRNA levels) most notably in the dentate gyrus granular cell layer of the hippocampus in adult rats (Nibuya et al., 1995, 1996; Malberg et al., 2000) and mice (Santerelli et al., 2003). In addition, animal studies have shown that neurogenesis can be decreased by a variety of stimuli (aging; various stressors; glucocorticoids), while antidepressant drugs are able to reverse the effects (Duman et al., 2001). More recently, it was shown that chronic fluoxetine treatment accelerates the maturation and functional integration of newborn, immature neurons in the dentate gyrus in wild-type SvEv129 adult male mice (Wang et al., 2008).

Thus, a positive regulation of 5-HT on the expression of the gene coding for BDNF may occur in adult hippocampus. At post-synaptic levels, a growth factor, BDNF requires activation of the high-affinity protein kinase receptor family TrkB (Tropomysine-related kinase B) to exert its biological effects. However, the actual knowledge regarding the relationship between BDNF and serotonin (5-HT) in the hippocampus is limited. For example, is there any reciprocal effect of BDNF on 5-HT neurotransmission? To answer this question, we have developed a dual experimental strategy by inducing either a decrease or an increase in BDNF protein levels in mouse brain.

First, we studied the SSRI response in heterozygous BDNF+/– mice, in which brain BDNF protein levels are decreased by half (Korte et al., 1995). These constitutive mutants develop enhanced inter-male aggressiveness and hyperphagia accompanied by significant weight gain in early adulthood. These behavioral abnormalities are known to correlate with 5-HT dysfunction (Lyons et al., 1999). In the present review, we summarized our data obtained in these mutant mice.

Second, we increased BDNF protein levels by using its local infusion into adult hippocampus in wild-type mice. Indeed, it was already shown that BDNF increases activity of brain monoaminergic systems in rats (Stuciak et al., 1996). Consistent with these preliminary findings, it has been previously reported that intra-hippocampal BDNF injection induces a dose-dependent antidepressant-like effect in rats that was observed 3 days and lasted up to 10 days after its bilateral injection (Shirayama et al., 2002). In awake freely moving mice, an acute intra-hippocampal injection of BDNF decreased basal and KCl-evoked release of 5-HT in adult hippocampus, as measured using microdialysis (Bennmansour et al., 2008). Interestingly, these effects of BDNF were blocked by the non-selective antagonist of TrkB receptors, K252a (Dethell et al., 2007; Bennmansour et al., 2008). Furthermore, BDNF potentiated the effects of an acute systemic SSRI administration or that of locally applied citralopram injection on dialysate 5-HT levels in the ventral hippocampus of adult mice.

Here, we extended this approach by studying the behavioral consequences of the intra-hippocampal BDNF injection combined with a systemic SSRI administration.

1.1. First strategy: experiments in constitutive BDNF heterozygous+/– mice

To study the relationship between BDNF and 5-HT neurotransmission in the hippocampus, we used adult BDNF+/– mice. We assessed the 5-HT reuptake capacity of the selective transporter SERT in vitro and in vivo. We reasoned that, if BDNF reduction plays a pivotal role in depression, a constitutive decrease in hippocampal BDNF in mutant mice would alter the efficacy of SSRI treatment.

The first BDNF mutant mice were generated by Ernfors et al., in 1994 because this member of the neurotrophin family can prevent the death of particular peripheral sensory neurons and of central motor neurons as well as dopaminergic and cholinergic neurons of the basal forebrain during development. Due to the early postnatal lethality of BDNF null mice, constitutive heterozygous BDNF+/– mice (Korte et al., 1995) or mice lacking its main TrkB receptor (Saarelainen et al., 2003) were used. Mice over-expressing the truncated isoform of the TrkB receptor were also generated (Saarinen et al., 2005; Rantamäki et al., 2007) as a supplementary model of blunted BDNF neurotransmission. Results obtained with conditional KO mice with floxed BDNF alleles allowing spatial and temporal regulation of BDNF deletion only appeared later on (Rios et al., 2001).

Young adult heterozygous BDNF+/– mice generated on a 129 Sv genetic background exhibit alterations in the expression of several 5-HT receptor mRNA particularly in the cortex, hippocampus, and hypothalamus (Lyons et al., 1999). Thus, from this pioneer study, we have learned that endogenous BDNF is critical for the normal development and function of central 5-HT neurons and for the elaboration of behaviors that depend on these nerve cells. Proliferation of adult progenitors and survival of immature neurons are significantly decreased in BDNF+/– mice (Lee et al., 2002). Therefore, BDNF+/– mice provide a useful model to study human psychiatric disorders related to dysfunction of serotonergic neurons.

We have already shown that constitutive BDNF+/– mice have increased basal extracellular 5-HT levels in the hippocampus associated with a decreased 5-HT reuptake capacity: these data have been published by Guiard et al. (2008). The following main results were obtained.

1.1.1. Basal extracellular 5-HT levels with conventional intracerebral microdialysis in mutant BDNF+/– mice

Conventional intracerebral microdialysis technique suggests that constitutive decreases in BDNF expression produced an elevation in basal dialysate 5-HT levels in the ventral hippocampus (Table 1). In addition, dialysate levels of its major metabolite, 5-hydroxy-indoleacetic acid (5-HIAA) were significantly reduced in the ventral hippocampus in BDNF+/– mice compared to BDNF+/+ mice (p < 0.05).

1.1.2. 5-HT transporter activity; [3H]-5-HT uptake in hippocampal synaptosomes from BDNF+/+ versus BDNF+/– mice

In vitro [3H]-5-HT uptake by synaptosomes prepared from the hippocampus was decreased in BDNF+/– mice compared to wild-type mice. Data are means ± SEM expressed as fmol/sample and pmol/sample for 5-HT and 5-HIAA, respectively. Data are means ± SEM expressed as fmol/20 μl for 39–46 determinations per group. *p < 0.01 and **p < 0.001 compared to wild-type control mice. From Guiard et al. (2008).

### Table 1

<table>
<thead>
<tr>
<th>BDNF+/+ wild-type mice</th>
<th>BDNF+/– mutant mice</th>
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<tr>
<td>5-HT (fmol/sample)</td>
<td>4.2 ± 0.2</td>
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<tr>
<td>5-HIAA (pmol/sample)</td>
<td>1.8 ± 0.2</td>
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Data are means ± SEM expressed as fmol/sample and pmol/sample for 5-HT and 5-HIAA, respectively. Data are means ± SEM expressed as fmol/20 μl for 39–46 determinations per group. *p < 0.01 and **p < 0.001 compared to wild-type control mice. From Guiard et al. (2008).
BDNF+/+ mice (Fig. 1). Constitutive reductions in BDNF significantly affected \( V_{\text{max}} \) (528 ± 32 versus 942 ± 59 pmol/mg protein per min in BDNF+/- mice and BDNF+/+ mice, respectively, \( p = 0.007 \)), but not \( K_m \) values for \([^{3}H]-5\)-HT uptake.

1.1.3. Paroxetine-induced changes in hippocampal 5-HT levels in mutant BDNF+/- mice

In the ventral hippocampus, a systemic administration of paroxetine (8 mg/kg, i.p.) increased extracellular 5-HT levels in BDNF+/- mice, but produced a smaller non-significant effect in BDNF+/- mice (Fig. 2A). Interestingly, we also reported that the neurochemical effects of paroxetine did not differ between BDNF+/- and BDNF+/+ mice in the frontal cortex (Fig. 2B) and dorsal raphe nucleus (Fig. 2C), both regions expressing SERT protein.

1.1.4. \([^{3}H]\)-citalopram binding site densities to hippocampal slices in mutant BDNF+/- mice

Autoradiographic study revealed a significant reduction in the number of \([^{3}H]\)-citalopram binding sites in the ventral hippocampus of BDNF+/- mutants compared to BDNF+/+ mice. In particular, a significant decrease in \([^{3}H]\)-citalopram binding sites was measured in the CA3 (\( p < 0.01 \), Table 2), but not in the dentate gyrus and CA1 (\( p > 0.05 \)) sub-regions of the hippocampus in BDNF+/- mutants compared to BDNF+/+ mice. No differences in the density of the labeling were noted in other brain regions such as frontal cortex, striatum and raphe nuclei with respect to genotype.

1.1.5. 5-HT transporter expression: SERT mRNA levels in the brain stem of BDNF+/+ versus BDNF+/- mice

Measurements of SERT mRNA transcripts in the brain stem of BDNF+/+ controls and heterozygous BDNF+/- mice revealed that no significant differences in SERT mRNA levels occurred in the brain stem between the two genotypes (Deltheil et al., 2007).

Taken together, these data suggest that an enhancement of basal extracellular 5-HT levels (5-HTText) induced a down-regulation of SERT, i.e., a decrease in its capacity to reuptake 5-HT, at serotonergic nerve terminals located in the hippocampus of adult BDNF+/- mice. 5-HT neurotransmission seems to be regulated by BDNF in a region-dependent manner. Results obtained by using chromoamperometry confirmed that 5-HT clearance rate increased markedly with age, and suggests that the profoundly reduced ability of 5- and 10-month-old BDNF+/- mice to clear 5-HT may be due to functional deficits in SERT, e.g., in the machinery/signaling required for insertion of SERTs into the plasma membrane and/or

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Fig. 1. \([^{3}H]\)-5-HT uptake in vitro in hippocampal synaptosomes from BDNF+/- versus BDNF+/+ mice. Scatchard analysis of the uptake of \([^{3}H]\)-5-HT into hippocampal synaptosomes of BDNF+/- mice and BDNF+/+ mice. Non-specific \([^{3}H]\)-5-HT uptake was determined in the presence of 2 μM of citalopram (n = 5 mice per group). Constitutive reductions in BDNF levels affected \( V_{\text{max}} \) (528 ± 32 versus 942 ± 59 pmol/mg protein per min in BDNF+/+ mice and BDNF+/+ mice, respectively, \( p = 0.007 \)), but were without significant effect on \( K_m \) values for \([^{3}H]\)-5-HT uptake (35 ± 3 versus 59 ± 11 nM in BDNF+/- mice and BDNF+/+ mice, respectively; \( p > 0.05 \)). From Guiard et al. (2008).

Fig. 2. Region-selective effects of paroxetine on 5-HT outflow in wild-type versus BDNF+/- mice. Area under the curve values (AUC; mean ± SEM) calculated for the amount of 5-HT outflow collected during the 0–180 min post-treatment period are expressed as percentage of mean values from saline-injected mice. A: Ventral hippocampus; B: frontal cortex and C: dorsal raphe nucleus of \( *p < 0.05 \) and \( **p < 0.01 \) relative to the corresponding saline-treated group (n = 7–8 animals per group). From Guiard et al. (2008).
activation of the SERT once it is positioned to take up 5-HT from extracellular fluid (Daws et al., 2007).

1.2. Second strategy: intra-hippocampal injection of BDNF in wild-type mice

In a second part of our study, BDNF protein levels were increased following its local infusion (100 ng) into adult hippocampus. We performed intracerebral microdialysis and behavioral experiments in wild-type mice.

1.2.1. Intracerebral microdialysis:

Effects of local intra-hippocampal BDNF injection on paroxetine-induced changes in hippocampal extracellular 5-HT levels in wild-type mice. BDNF potentiated the effects of a systemic administration of paroxetine (4 mg/kg, i.p.) on dialysate 5-HT levels in the adult ventral hippocampus in mice (Fig. 3; from Deltheil et al., 2007).

1.2.2. Behavioral tests

1.2.2.1. Effect of BDNF on paroxetine-induced antidepressant-like activity in the forced swim test (FST) in wild-type mice. Paroxetine, and also BDNF, increased the swimming duration in the FST in adult male Swiss mice (*p < 0.05 for both drugs). Co-administration of BDNF with paroxetine potentiated this effect (**p < 0.001 when compared to the effects of paroxetine alone; Fig. 4).

1.2.2.2. Effect of co-administration of BDNF with paroxetine in the open field paradigm. Mice treated acutely with paroxetine (4 mg/kg) or vehicle followed by an intra-hippocampal BDNF (100 ng) injection were tested in the open field (OF) to assess their anxiety-like state. BDNF induced an anxiogenic-like profile since a significant decrease in the time spent in the center was measured (p < 0.001; Fig. 5). The anxiogenic-like effect after an intra-hippocampal BDNF injection did not affect the locomotor activity (data not shown). By contrast to paroxetine, the benzodiazepine diazepam (1 mg/kg, i.p. used here as a reference drug), displayed an anxiolytic-like activity since it induced an increase in the time spent in the center (p < 0.05). Finally, the anxiogenic-like effect of BDNF was prevented by a paroxetine pre-treatment since co-administration of paroxetine with BDNF did not affect the time spent in the center (p > 0.05).

2. Discussion and conclusion

The present study assessed whether a decrease (i.e., a constitutive deletion of one copy of the BDNF gene) or an increase (i.e., a local intra-hippocampal BDNF injection) in BDNF protein levels in the mouse brain can affect hippocampal 5-HT neurotransmission in adulthood. In both cases, decreasing or increasing BDNF protein levels led to selective alterations of 5-HT neurotransmission in the mouse adult ventral hippocampus.

Table 2

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<th>Ventral hippocampus</th>
<th>BDNF+/+ wild-type mice</th>
<th>BDNF+/- mutant mice</th>
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<tr>
<td>Dentate gyrus</td>
<td>34.6 ± 3.7</td>
<td>35.7 ± 4.6</td>
</tr>
<tr>
<td>CA1</td>
<td>37.1 ± 1.8</td>
<td>34.1 ± 2.5</td>
</tr>
<tr>
<td>CA3</td>
<td>74.4 ± 2.7</td>
<td>59.4 ± 3.7**</td>
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Data are expressed as mean ± SEM in fmol of [3H]-citalopram/mg tissue equivalent and represent means ± SEM of specific [3H]-citalopram binding. **p < 0.01 compared to wild-type control mice (n = 5 mice per group). From Guiraud et al. (2008).
First, heterozygous BDNF+/− mice (generated by Korte et al., 1995) display behavioral abnormalities known to correlate with 5-HT dysfunction (Lyons et al., 1999). We found, in these mutants, an enhancement of basal extracellular 5-HT levels. As revealed in vitro by using autoradiography and synaptosome techniques, one of the causes of this alteration could be a down-regulation of SERT, i.e., a decrease in its capacity to reuptake 5-HT. Indeed, [3H]-citalopram binding to SERT in the CA3 sub-region of the ventral hippocampus and [3H]-5-HT uptake in hippocampal synaptosomes, revealed a blunted SERT function (Guiard et al., 2008). In addition, intracerebral in vivo microdialysis demonstrated that, paroxetine-induced increase in hippocampal extracellular 5-HT levels was also reduced in BDNF+/− mice, while it produced robust effects in wild-type littermates (Guiard et al., 2008). Taken together, these in vitro and in vivo results demonstrate that SERT capacity to reuptake 5-HT in adult hippocampus is altered in BDNF+/− mice, suggesting that these mutant mice can be viewed as an animal model of genetic resistance to serotonergic antidepressant drugs.

Second, in wild-type BDNF+/+ mice, we examined neurochemical and behavioral consequences of an intra-hippocampal injection of BDNF (100 ng) combined with a systemic administration of an SSRI. By using conventional in vivo microdialysis technique, we showed that BDNF induced a rapid and transient increase in paroxetine response on dialysate 5-HT levels in the adult hippocampus. The potentiation of this effect by the [BDNF + SSRI] association had important behavioral consequences. Indeed, in the mouse FST, intra-hippocampal BDNF injection alone or combined with paroxetine decreased the total immobility duration. Furthermore, BDNF potentiated the effect of paroxetine on swimming behavior, which relies on the activation of the serotonergic neurotransmission (Page et al., 1999). These data suggest an interesting antidepressant-like activity of the [BDNF + SSRI] combination in correlation with the neurochemical effects on 5-HT neurotransmission in the hippocampus. To our knowledge, this is the first study showing that such a combined [BDNF + SSRI] treatment can improve an antidepressant-like activity. Our behavioral data are consistent with results showing that a single bilateral infusion of BDNF into the dentate gyrus or CA3 pyramidal cell layers, two subfields of the hippocampus, at a comparable dose used herein, elicited antidepressant-like effects in the rat FST, mainly through an increase in swimming behavior (Shirayama et al., 2002). By using a genetic model of mice over-expressing BDNF in excitatory neurons of the forebrain, it was also showed that BDNF transgenic mice displayed reduced total immobility time in the FST (Govindarajan et al., 2006).

Furthermore, in the OF paradigm, intra-hippocampal BDNF injection had a facilitatory effect on anxiety-like behavior, and paroxetine prevented this anxiogenesis in mice. Diazepam used as a positive control, effectively reduced anxiety in the OF test, while paroxetine alone had no effects. Such a BDNF-induced anxiogenesis was not reported by other groups working in rats (Shirayama et al., 2002). However, Govindarajan et al. (2006) found that mice over-expressing BDNF in the whole forebrain exhibit a facilitatory effect on anxiety-like behavior. The fact that intra-hippocampal injection of BDNF can induce anxiogenesis contrasts with the neurotrophic hypothesis of depression predicting that increases in brain BDNF should counteract the effects of chronic stress (Duman et al., 2001). However, the increased swimming behavior in the FST as observed in mice treated with [BDNF + paroxetine] as well as the potentiation of paroxetine response on 5-HT neurotransmission in microdialysis experiments are consistent with an antidepressant-like effect of BDNF, as proposed by the neurotrophic hypothesis.

Due to our experimental protocol (a stereotaxic injection of BDNF), the hippocampus and 5-HT likely participated in mediating both anxiety- and antidepressant-like behaviors of BDNF in mice. As suggested by Govindarajan et al. (2006), the neurotrophic factor can both facilitate anxiety-like symptoms and inhibit depressive symptoms through its differential actions locally in the hippocampus and amygdala, respectively. Hence, increasing hippocampal BDNF concentrations in the presence of a SSRI should prevent symptoms associated with depression. This suggests that behavioral responses to BDNF in the FST and OF in wild-type mice involved other sites of action into the brain, thus activating a broader brain circuit and serotonergic pathways. Shirayama et al. (2002) reported a limited diffusion of BDNF in rat hippocampus (0.5 mm). Thus, a direct relationship between BDNF on 5-HT neurotransmission in the hippocampus is likely to have occurred to explain responses to the behavioral tests.

However, an indirect mechanism involving TrkB receptors located in the raphe nuclei could also explain the behavioral effects of BDNF injection. A long post-synaptic 5-HT feedback mechanism could have influenced these behavioral responses. Indeed, the existence of a complex regulation of dorsal raphe serotonergic neurons by the medial prefrontal cortex afferents was already described (Celada et al., 2001). The stimulus-induced excitation of some 5-HT neurons by descending excitatory fibers releases 5-HT, which inhibits the same or other DR neurons by acting on 5-HT1A autoreceptors. Afferents from the cortex also inhibit 5-HT-5HT-5HT-5HT neurons through the activation of GABAergic interneurons. Ascending serotonergic pathways may control the activity of this descending pathway by acting on post-synaptic 5-HT1A receptors. Similarly, such a descending pathway may also exist for the control of various raphe 5-HT neurons by forebrain regions (Bosker et al., 1997; Celada et al., 2002; Hajós et al., 2003).

In situ hybridization experiments for TrkB receptor mRNA demonstrate that the full-length of TrkB receptors is expressed throughout the ventral hippocampus in the different layers of the dentate gyrus, but also in the hilus as well as in the dorsal raphe nucleus (DRN) in which the cell bodies of the serotonergic neurons are located (Drs K. Tanaka and R. Hen, data not shown). In addition, TrkB receptor mRNA is co-expressed with 5-HT1A receptor mRNA in the DRN. These data raise the possibility that the neurochemical and behavioral effects of intra-hippocampal BDNF injection can mobilize both pre- and post-synaptic elements of the brain 5-HT neurotransmission.

The relative opposition of neurochemical and behavioral responses obtained from adult mouse following either a decrease (in BDNF+/− mutant mice) or an increase in BDNF (bilateral intra-hippocampal injection) should hold our attention. Table 3 summarizes the results.
obtained by using the two complementary strategies. Although the two approaches gave very interesting information, they are not strictly comparable. Indeed, heterozygous BDNF+/− mice used in our first strategy were generated by homologous recombination (Korte et al., 1995), thus are constitutive mutants. By contrast, in the second strategy, a single bilateral intra-hippocampal injection of BDNF was performed in wild-type mice. Thus, the best comparison would be between adult BDNF+/− mice and adult wild-type mice chronically treated with BDNF. Another possibility could be to study changes in 5-HT concentrations and the extraction fraction of 5-HT (Ed), which provides an in vivo index of 5-HT uptake in the ventral hippocampus of BDNF+/− and wild-type mice. In the ZNF method, four samples were collected to determine basal hippocampal 5-HT levels before local perfusion of increasing concentrations of 5-HT (0, 5, 10 and 20 nM: Fig. 6). The dialysate 5-HT concentrations (Cout) obtained during perfusion of the various concentrations of 5-HT (Cin) were used to construct a linear regression curve for each animal (Guiraud et al., 2008; Deltheil et al., 2007). The net change in 5-HT (Cout−Cin) was plotted on the y-axis against Cin on the x-axis. Extracellular 5-HT levels [5-HT]ext and the extraction fraction of the probe (Ed) were determined as described by Parsons et al. (1991). The concentration of 5-HT in the extracellular space [5-HT]ext is estimated from the concentration at which Cin−Cout = 0 and corresponds to point at which there is no net diffusion of 5-HT across the dialysis membrane. The extraction fraction (Ed) is the slope of the linear regression curve and has been shown to
provide an estimate of changes in transporter-mediated 5-HT uptake (Gardier et al., 2003; Parsons et al., 1991).

First, the ZNF method of quantitative microdialysis confirmed our in vitro data by showing that BDNF+/− heterozygous mice have a decreased 5-HT reuptake capacity associated with increased basal extracellular 5-HT levels in the hippocampus (Fig. 7A). Thus, basal extracellular 5-HT levels in the ventral hippocampus were significantly higher in BDNF+/− mice.

![Graph A](image1)

**Fig. 7.** Zero net flux analysis of 5-HT levels in the ventral hippocampus of BDNF+/+ versus BDNF+/- mice. The plots show the means ± SEMs gain or loss of 5-HT (Cin–Cout) as a function of Cin (0, 5, 10, 20 nM of 5-HT) and the average linear regression of the data A: in wild-type BDNF+/+ mice (-----) versus mutant BDNF+/- heterozygous mice (____); B: in wild-type mice treated with either saline (-----) or an intra-hippocampal BDNF 100 ng injection (____). The Cin at which Cin–Cout = 0 equals the extracellular 5-HT levels ([5-HT]ext), and the slope of linear regression corresponds to the extracellular fraction of the probe (Ed). The y-intercept corresponds to theoretical dialysate 5-HT levels that would be obtained in a conventional dialysis experiment. (b) Means ± SEM of [5-HT]ext, i.e., basal 5-HT release; (c) means ± SEM of the slope Ed, i.e., 5-HT uptake in vivo. Number of mice n = 10–12 per group. *p < 0.05 and **p < 0.01 when compared to wild-type controls.
compared to wild-type mice (**p < 0.05). Previous studies have shown that manipulations that decrease neurotransmitter uptake also decrease the recovery of neurotransmitter from the tissue as reflected in the extraction fraction, Ed (Parsons et al., 1991). In agreement with an elevated basal extracellular 5-HT concentrations, BDNF+/− mice exhibited a significantly lower Ed compared to wild-type mice. Thus, a reduced BDNF gene expression in heterozygous BDNF+/− mice led to a decreased hippocampal SERT activity, and consequently to an increase in basal extracellular 5-HT levels at serotonergic nerve terminals in the adult hippocampus. This increase in hippocampal 5-HT neurotransmission was selective since it was not detected in other brain regions such as the frontal cortex and striatum in adult heterozygous BDNF+/− mice (Szapcs et al., 2004). This hypothesis was in agreement with the absence of effects of paroxetine, on dialysate 5-HT levels in the ventral hippocampus (Fig. 2A). Constitutive decreases in brain BDNF levels alter the effects of paroxetine in the ventral hippocampus, but neither in the frontal cortex nor in the vicinity of cell bodies of 5-HT neurons, i.e., in the dorsal raphe nucleus (Fig. 2B and 2C, respectively), strongly supporting the region-selective alteration of SERT in BDNF+/− mice (Guirard et al., 2008). A reduction in SERT function in the adult hippocampus (Guirard et al., 2008) rather than in SERT mRNA expression in the brain stem (Deltheil et al., 2007) occurred in these mutant mice. These results suggest that BDNF is necessary for an appropriate uptake of 5-HT at serotonergic nerve terminals in the hippocampus of adult mice.

Second, Regarding the effect of BDNF on the same paradigm, the ZNF method, surprisingly, we found that its intra-hippocampal injection decreased the function of SERT function in wild-type mice as observed in heterozygous BDNF+/− mice. However, the decreased capacity of SERT to reuptake 5-HT was not associated to an increase in basal 5-HTx in the hippocampus of WT mice (Fig. 7B). Furthermore, when 5-HT uptake sites were measured using [3H]-cyanoimipramine following intra-cerebroventricular injection of BDNF, it was found that the specific binding in the CA3 area of the hippocampus was similar in rats treated with vehicle and in those treated with BDNF (Bennamour et al., 2008). Thus, the two different experimental approaches led to a similar alteration in SERT function, but the underlying mechanisms are likely different.

Despite these results and the use of constitutive KO mice, further investigations are required to determine precisely (i) the brain region(s) where BDNF mediates its excitatory effects on 5-HT neuronal system, and neuronal circuits involved in this effect, (ii) whether this neurotrophic factor plays a major role in the regulation of 5-HT during development and/or in adulthood. Conventional knockout technology has limitations, such as lethal phenotype or when gene function at a certain developmental stage must be elucidated (Alia and Nakao, 2007).

All together, these results suggest that a poly-therapy combining BDNF with a chronic SSRI treatment could lead to faster neurochemical and behavioral benefits by limiting the anxiogenic effect of endogenous BDNF. It provides a better knowledge of the complex relationship between BDNF and 5-HT neurotransmissions in the hippocampus. It may help to understand better the physiopathology of depression, thus allowing to improve its treatment in a near future.

Acknowledgments

D.T. C. were recipient of a fellowship from “Ministère de l’Éducation Nationale, de l’Enseignement Supérieur et de la Recherche” (MENESR, Paris, France) during the performance of this work.

This work has been supported by the technical assistance of the Animal care facility of the “Institut Fédératif de recherche-IFR141” of the Paris XI University.

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