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Review

Consequences of changes in BDNF levels on serotonin neurotransmission, 5-HT transporter expression and function: Studies in adult mice hippocampus

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Abstract

In vivo intracerebral microdialysis is an important neurochemical technique that has been applied extensively in genetic and pharmacological studies aimed at investigating the relationship between neurotransmitters. Among the main interests of microdialysis application is the infusion of drugs through the microdialysis probe (reverse dialysis) in awake, freely moving animals. As an example of the relevance of intracerebral microdialysis, this review will focus on our recent neurochemical results showing the impact of Brain-Derived Neurotrophic Factor (BDNF) on serotonergic neurotransmission in basal and stimulated conditions. Indeed, although the elevation of 5-HT outflow induced by chronic administration of selective serotonin reuptake inhibitors (SSRIs) causes an increase in BDNF protein levels and expression (mRNA) in the hippocampus of rodents, the reciprocal interaction has not been demonstrated yet. Thus, the neurochemical sight of this question will be addressed here by examining the consequences of either a constitutive decrease or increase in brain BDNF protein levels on hippocampal extracellular levels of 5-HT in conscious mice.

Keywords: Antidepressant drugs; Serotonin; BDNF; Neurogenesis; Genetically modified animals; Conventional microdialysis; Zero net flux method of quantitative microdialysis

Contents

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1. Introduction

Most of the antidepressants such as Selective Serotonin Reuptake Inhibitors (SSRI) act as indirect agonists of monoamine receptors. While 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. This gap in timing between SSRI near-immediate effect on neurotransmitter systems and the slow symptomatic recovery is a paradox that has not been completely solved yet. At presynpatic level, SSRI-induced blockade of SERT results in a rapid suppression of the firing activity of 5-HT neurons in the brainstem (Blier, 2001). Consequently, despite the 5-HT reuptake inhibition also taking place at nerve terminals, there is a decrease in 5-HT release via activation of 5-HT1A (somatodendritic) or 5-HT1B (nerve terminal) autoreceptors (Rutter et al., 1995). Thus, depending on the brain area, only a small increase or no change at all in the synaptic availability of 5-HT occurs (Romero et al., 1996; Malagie et al., 1996). As the treatment is prolonged, a robust and time-dependent downregulation of the 5-HT transporter SERT is observed (Pineyro et al., 1994; Benmansour et al., 2002), while 5-HT$_{1A}$ autoreceptors gradually desensitize leading to a progressive recovery to normal of the firing rate of 5-HT neurons as well as to an increased 5-HT neurotransmission in synpases (Blier et al., 1986; Chaput et al., 1986; El Mansari et al., 2005). At post-synaptic levels, a growth factor, the Brain-Derived Neurotrophic Factor (BDNF), requires activation of the high-affinity protein kinase receptor family TrkB (Tropomyosine-related kinase B) to exert its biological effects. The properties of BDNF are different according to the brain region studied: for example, it regulates synaptic plasticity in the adult visual cortex (Tsanov and Manahan-Vaughan, 2007). In addition, BDNF regulates lipid biosynthesis (Suzuki et al., 2007). In the adult hippocampus, BDNF might be involved in this delay of onset of SSRI. Indeed, chronic, but not acute, SSRI treatment by increasing 5-HT neurotransmission causes an increase in BDNF protein levels and expression (mRNA) most notably in the dentate gyrus granular cell layer of the hippocampus in adult rats (Nibuya et al., 1995, 1996) and mice (Santarelli et al., 2003). Thus, a positive regulation of 5-HT on the expression of the gene coding for BDNF may occur in adult hippocampus. These effects could be related to increases in neurogenesis, i.e., ability of progenitors cells to differentiate into neurons or glia cells (Malberg et al., 2000). This cascade of events may contribute to the therapeutic effects of antidepressant drugs. 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.

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 BDNF+/−mice 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).

Second, we increased BDNF protein levels by its local infusion into adult hippocampus by reverse microdialysis in wild-type mice. Indeed, it was found that BDNF increases activity of brain monoaminergic systems in rats (Siuciak et al., 1996). BDNF infusion into the forebrain results in an elevation of 5-HT neuronal fiber density and also protects serotoninergic neurons from neurotoxic damage in rats (Mamounas et al., 1995). In another study, intra-hippocampal BDNF injection induces an antidepressant-like effect in rats that was dose-dependent (a dose as low as 0.25 μg of BDNF induced it), was observed 3 days and lasted up to 10 days after its bilateral injection (Shirayama et al., 2002).

In the present study, both *in vivo* conventional and quantitative intracerebral microdialysis studies have been performed in these two animal models, and we measured extracellular levels of 5-HT in the adult hippocampus of awake, freely moving mice.

2. Microdialysis: principles and methodology

2.1. Mice

Male wild-type BDNF+/+and heterozygous mutant BDNF+/− mice (3 to 4 months of age and 25–30 g in body weight) were bred on a mixed S129/Sv×C57BL/6 genetic background (Korte et al., 1995) and raised at the animal facility of the university of Paris XI (Chatenay-Malabry, France). Heterozygous adult mice with one functional BDNF allele (BDNF+/−) exhibited reduced BDNF protein levels in the hippocampus (data not shown). All animals were genotyped by polymerase chain reaction (Guiard et al., 2007).

For local BDNF injection, adult male 129S6/SvEvTac wild-type mice (Taconic Farms, Ry, Denmark) were used. All mice were 7–8 weeks old, weighed 23–25 g, and were housed in groups of 6 mice per cage under standard conditions (12:12 h light–dark cycle, 22±1 °C ambient temperature, 60% relative humidity, food and water *ad libitum*).

2.2. Conventional intracerebral microdialysis

Concentric dialysis probes (0.30 mm outer diameter) were constructed of cuprophane and set up as described previously (Guiard et al., 2004; Malagie et al., 2001). Probes were implanted into the ventral hippocampus (active length of 1.6 mm) in anesthetized mice (chloral hydrate, 400 mg/kg, intraperitoneally, i.p.) according to the mouse brain atlas of Paxinos and Franklin (2001). The stereotaxic coordinates from Bregma (in mm) were: $A=-3.4$, $L=3.4$, $V=4.0$. The next day, after recovery from surgery, probes were continuously perfused with artificial cerebrospinal fluid (aCSF) in awake animals at a flow rate of 1.5 µl/min. Dialysate samples were collected every 15 min for the measurement of 5-HT by using high-performance liquid chromatography coupled to an amperometric detector (1049A, Hewlett-Packard, Les Ulis, France). The limit of sensitivity for 5-HT was ~0.5 fmol/sample (signal-to-noise ratio 2). After 1 h of stabilization necessary to reach uniform concentrations of 5-HT in dialysates, four samples were collected to measure basal extracellular 5-HT values. Drugs were then injected intraperitoneally (paroxetine 4 and/or 8 mg/kg) at $t=0$ and subsequent dialysate fractions were collected. BDNF was dissolved in artificial cerebrospinal fluid (aCSF) (composition in mM: NaCl 147, KCl 3.5, $CaCl_2$ 1.26, $MgCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 25.0, pH 7.4±0.2) and administered locally into the ventral hippocampus (vHi) *via* a silica catheter glued to the microdialysis probe [(0.2 µL/min for 2 min by using a Picoplus microinjector (Harvard Apparatus, Les Ulis, France)], at the dose of 20 and 100 ng. The tyrosine kinase inhibitor, K252a (10 µM) was dissolved in aCSF containing 0.1% of dimethyl sulfoxide (DMSO), respectively, and was locally perfused by 'reverse microdialysis' at a flow rate of 1.5 µL/min. For each experiment, control group received the appropriate vehicle.

2.3. Zero net flux method of quantitative intracerebral microdialysis

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). The dialysate 5-HT concentrations

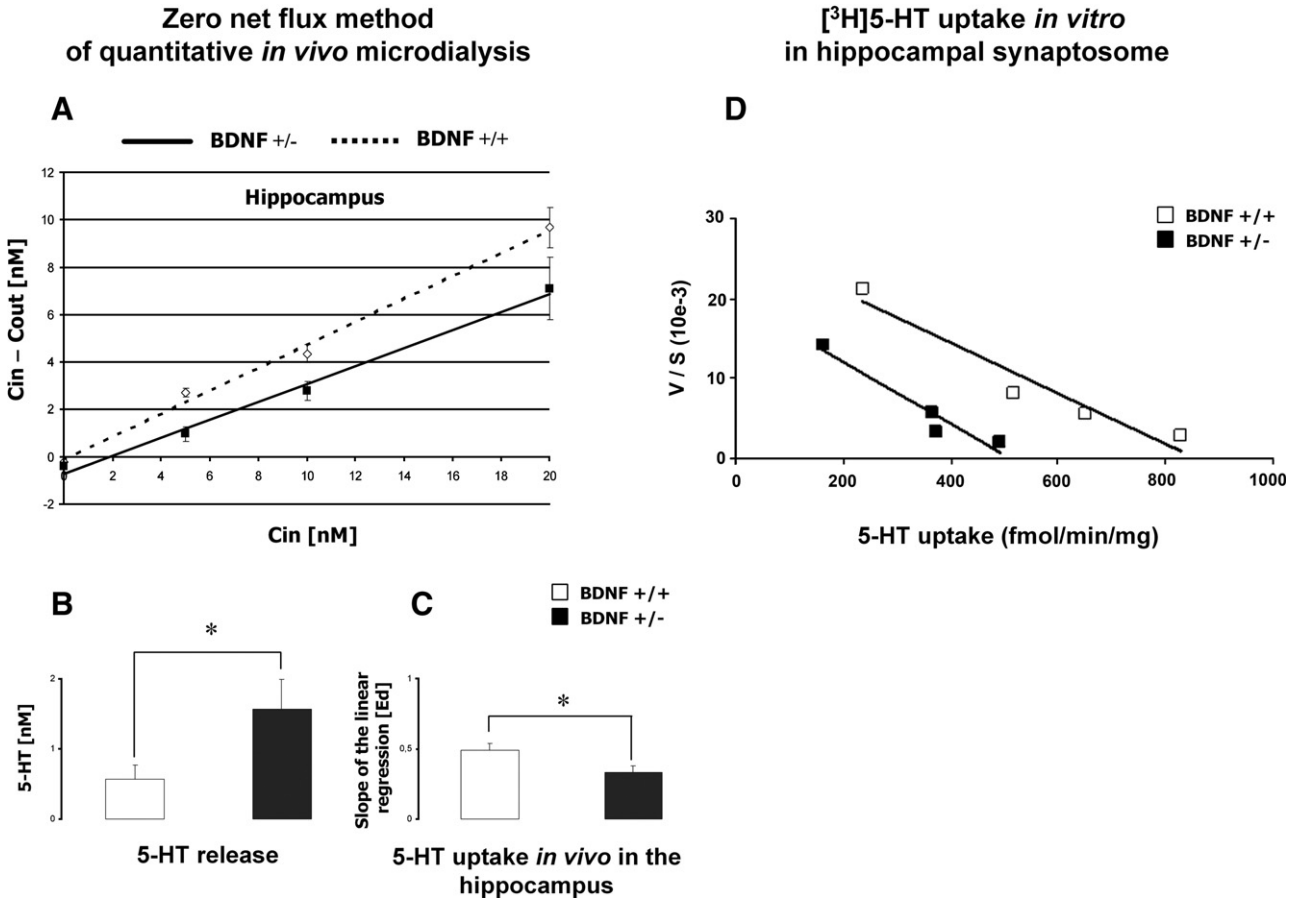

Fig. 1. Zero net flux analysis of 5-HT levels in the ventral hippocampus of BDNF+/+versus BDNF+/−mice. The plots in (A) show the means±SEMs gain or loss of 5-HT (Cin–Cout) as a function of Cin (0, 5, 10, 20 nM of 5-HT) and the average linear regression of the data in BDNF+/−mice and BDNF+/+mice. The 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 (E_d). The *y*-intercept corresponds to theoretical dialysate 5-HT levels that would be obtained in a conventional dialysis experiment. Statistically significant differences were observed between the two genotypes studied regarding B: means±SEM of [5-HT]ext, i.e., basal 5-HT release; and C: means±SEM of the slope E_d i.e., 5-HT uptake *in vivo*. Number of mice $n=10$–12 per group. *$P<0.05$ compared to wild-type controls. D: [^{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).

(Cout) obtained during perfusion of the various concentrations of 5-HT (Cin) were used to construct a linear regression curve for each animal (Guiard et al., 2007). The net change in 5-HT (Cin–Cout) 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 a 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).

2.4. Statistical analysis

All data are reported as means ± SEMs. Following linear regression of the data for each animal in the zero net flux microdialysis experiments, unpaired two-tailed Student's *t*-tests were used to assess the effects of genotype on extracellular levels of 5-HT in the hippocampus and Ed. For conventional microdialysis experiments (paroxetine i.p.; BDNF injection), statistical analyses were performed on areas under the curve (AUC) values for the amount of 5-HT outflow collected during the 0–120 min post-treatment period. To compare different AUC values in each group of mice, a one-way ANOVA with treatment factor followed by Fischer protected least significance difference (PLSD) *post-hoc* test was conducted. In addition, basal 5-HT levels in the ventral hippocampus across groups of mice involved in conventional microdialysis studies have been compared by using a Student's *t*-test.

For the 5-HT uptake experiments performed in hippocampal synaptosomes, results were analyzed by non-linear regression and the uptake capacity (V_{max}) and K_m of [^{3}H]-5-HT were calculated. Then, two-way ANOVA was performed with 5-HT concentration as a within-subject variable and genotype as a between-subject variable.

For [^{3}H]-citalopram autoradiography study, the optical density of selected brain region was measured and converted into fmol/mg tissue using the standard curve. Non-specific binding was subtracted from total binding to evaluate specific binding in each brain region of each animal. Measurements were made on three sections from each brain region, and the values were averaged for each animal. The values for each region for each animal were then analyzed by Student's *t*-test for differences between genotypes.

Conventional *in vivo* microdialysis

Basal dialysate 5-HT and 5-HIAA levels in the ventral hippocampus across all BDNF +/+ and BDNF +/- mice studied with the conventional microdialysis technique. Data are means ± SEM expressed as fmol or pmol/sample

	BDNF +/+ Wild-type mice	BDNF +/- mutant mice
5-HT (fmol/sample)	4.2 ± 0.2	7.9 ± 0.7***
5-HIAA (pmol/sample)	1.8 ± 0.2	0.9 ± 0.2**

Each value is a mean ± SEM. 39 to 46 determinations per group. **p<0.01 and ***p<0.001 compared to wild-type control mice.

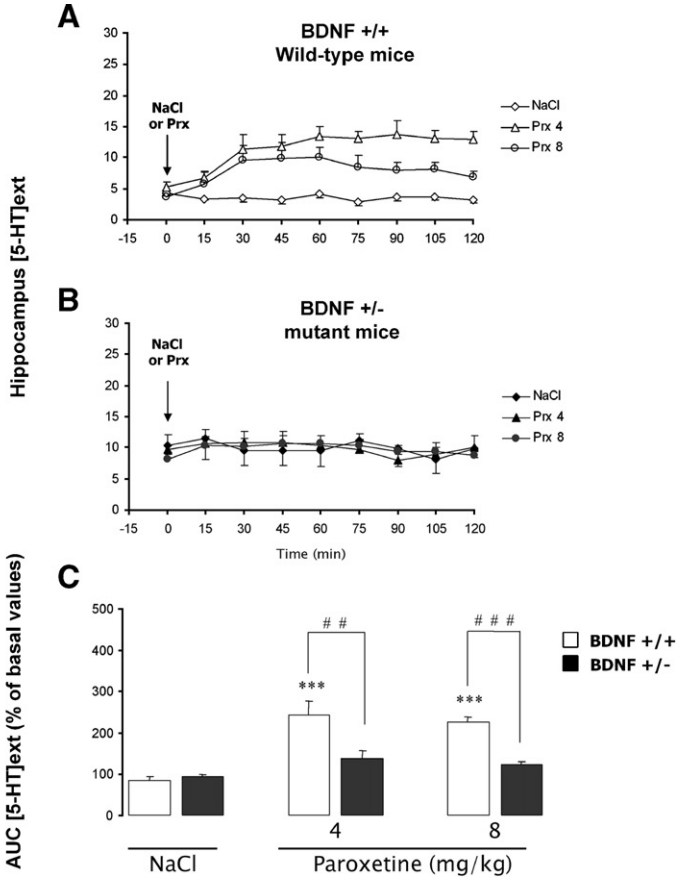

Fig. 2. Dose-response effects of paroxetine on 5-HT outflow in the ventral hippocampus of BDNF+/+versus BDNF+/−mice. Table: Basal extracellular 5-HT and 5-HIAA levels as measured by conventional intracerebral microdialysis in mice. A, B and C: Effects of an acute dose of paroxetine on dialysate 5-HT levels in the ventral hippocampus of wild-type mice and BDNF+/−mice. Data are means ± SEM of extracellular 5-HT levels expressed as fmol/samples in A: BDNF+/+(white symbols) and B: BDNF+/−mice (black symbols) following exposure to (□ or ■) saline or (○ or ●) paroxetine (Prx) 4 mg/kg or (△ or ▲) 8 mg/kg, respectively. C: 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. ***P<0.001 relative to the corresponding saline-treated group; ##P<0.01 and ###P<0.001 relative to BDNF+/+mice. (n=7–8 animals per group).

For all data, significant level was set at $P \leq 0.05$. All analyses were conducted using a Statview 5.0 (JMP Software, Cary, NC).

3. Strategy 1: effects of decreasing BDNF levels on 5-HT neurotransmission in the hippocampus. Comparison of BDNF+/+versus adult BDNF+/−mice

3.1. Basal levels with the zero net flux method of quantitative microdialysis

The zero net flux method of quantitative microdialysis was used to evaluate basal extracellular 5-HT levels in the ventral hippocampus of BDNF+/−and BDNF+/+mice. In a recent report, we demonstrated that the extracellular 5-HT levels corrected for *in vivo* recovery were significantly higher in BDNF+/−mice compared to wild-type mice (Fig. 1A) (Guiard et al., 2007). Thus, constitutive deletion of a single copy of the BDNF gene is associated with an increase in basal 5-HT levels in the ventral hippocampus. This effect may reflect either increase in hippocampal 5-HT release and/or decrease in 5-HT uptake *in vivo*. 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 Fig. 1B and C).

3.2. 5-HT transporter activity: [3H]5-HT uptake in hippocampal synaptosomes from BDNF+/+versus BDNF+/−mice

In vitro [^{3}H]-5-HT uptake by synaptosomes prepared from the hippocampus was decreased in BDNF+/−mice compared to BDNF+/+mice (Fig. 1D). Constitutive reductions in BDNF affected V_{max} (528±32 *vs.* 942±59 pmol/mg protein per min in BDNF+/−mice and BDNF+/+mice, respectively, $P=0.007$), but was without significant effect on K_m values for [^{3}H]-5-HT uptake (35±3 *vs.* 59±11 nM in BDNF+/−mice and BDNF+/+ mice, respectively, $P>0.05$; Guiard et al., 2007).

3.3. Basal extracellular 5-HT levels with conventional intracerebral microdialysis in mice

Conventional microdialysis data confirmed that constitutive decreases in BDNF expression also produce an elevation in basal dialysate 5-HT concentrations (Table in Fig. 2). In addition, dialysate levels of its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were significantly reduced in the ventral hippocampus in BDNF+/−mice compared to BDNF+/+mice ($P=0.01$; Guiard et al., 2007).

Table 1
Autoradiographic [^{3}H]-citalopram binding site densities in the subregions of the ventral hippocampus of BDNF+/+wild-type versus heterozygous BDNF+/−adult mice

Ventral hippocampus	BDNF+/+wild-type mice	BDNF+/-mutant mice
Dentate gyrus	34.6±3.7	35.7±4.6
CA1	37.1±1.8	34.1±2.5
CA3	74.4±2.7	59.4±3.7**

Data are expressed as mean±SEM in fmol of [^{3}H]-citalopram/mg tissue equivalent and represent means±SEM of specific [^{3}H]-citalopram binding. **$P<0.01$ compared to wild-type control mice ($n=5$ mice per group).

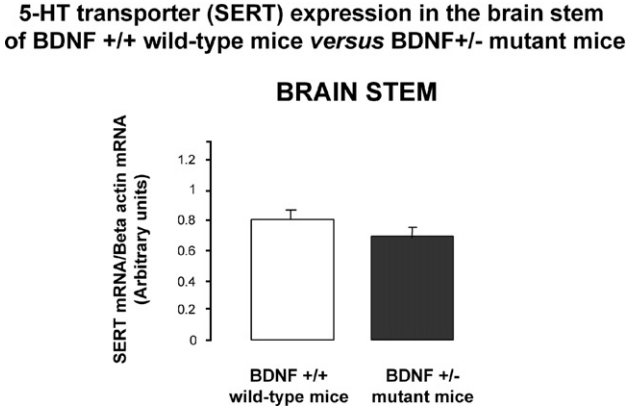

Fig. 3. SERT mRNA expression in the brain stem of BDNF+/+wild-type (white bar) and heterozygous BDNF+/−mice (black bar) as measured by quantitative real-time PCR. The values shown are means±SEM of SERT mRNA levels normalized to β-actin mRNA levels. Data from 7 mice per group.

3.4. Paroxetine-induced changes in hippocampal 5-HT levels in mice

In the ventral hippocampus of BDNF+/+mice extracellular 5-HT levels were increased by paroxetine administered at the dose of 4 mg/kg compared to the corresponding group of wild-type mice treated with vehicle (Fig. 2A and C). In BDNF+/− mice, extracellular 5-HT levels were not affected by paroxetine neither at 4 mg/kg nor at 8 mg/kg compared to the corresponding group of mutant mice treated with vehicle (Fig. 2B and C). Interestingly, the neurochemical effects of paroxetine did not differ between BDNF+/−and BDNF+/+mice in the frontal cortex and the dorsal raphe nucleus, both regions expressing SERT protein (Guiard et al., 2007).

3.5. [^{3}H]citalopram binding site densities to hippocampal slices in mutant BDNF+/−mice

To further explore the underlying causes of the decreases in extraction fraction in hippocampal 5-HT reported above for BDNF+/−mice, we performed autoradiography in the hippocampus of theses mutants. Examination of [^{3}H]-citalopram binding site densities 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 1), but not in the dentate gyrus and CA1 ($P>0.05$, Table 1) sub-regions of the hippocampus in BDNF+/−mutants compared to BDNF+/+mice. As well, no differences in the density of the labeling were noted in the other brain regions such as frontal cortex, striatum and raphe nuclei with respect to genotype (Guiard et al., 2007).

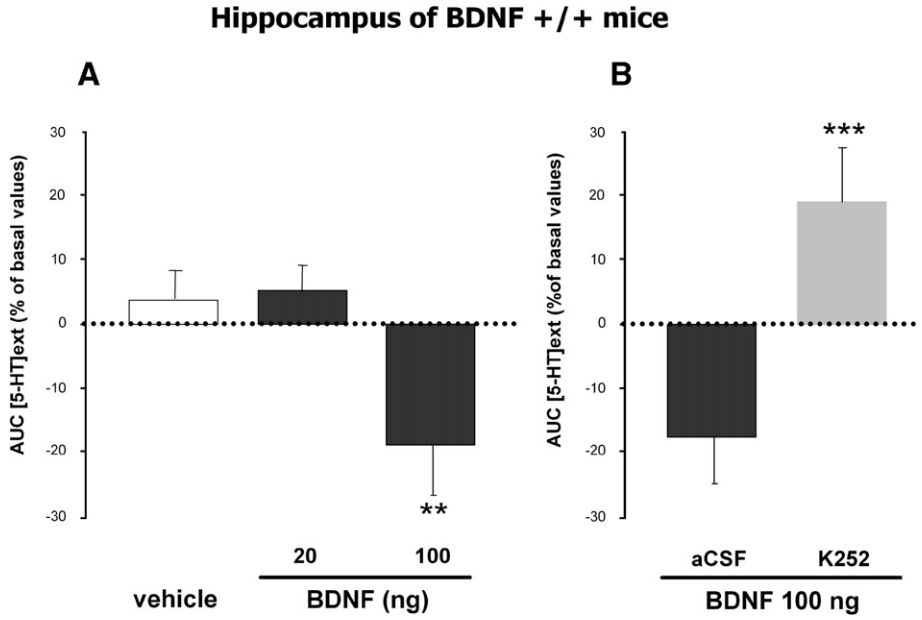

Fig. 4. Effects of intra-hippocampal perfusion of BDNF on dialysate 5-HT levels in the ventral hippocampus of freely moving wild-type mice. Area under the curve (AUC; mean±SEM) values calculated for the amount of 5-HT outflow in the hippocampus measured during the 0–120 min post-treatment with either vehicle or BDNF alone or BDNF (100 ng) in the absence (A) or the presence (B) of the neurotrophin receptor inhibitor K252a (10 μM). Data were expressed as percentages of baseline (7–11 mice per group). $^{**}P<0.01$; $^{***}P<0.001$ from corresponding vehicle-treated group (ANOVA, Fisher's PLSD *post hoc* test).

3.6. 5-HT transporter expression: SERT mRNA levels in the brain stem of BDNF+/+versus BDNF+/−mice

In the mouse brain, high levels of the 5-HT transporter (SERT) mRNA were detected in all brain stem raphe nuclei where serotonergic cell bodies are located (Bengel et al., 1997). To assess whether the down-regulation of SERT observed in the ventral hippocampus of BDNF heterozygous mice is linked to a decrease in SERT expression, we measured SERT mRNA transcripts in the brain stem of BDNF+/+controls and heterozygous BDNF+/−mice (Fig. 3). No significant differences in SERT mRNA levels have been found in the brainstem between the two genotypes.

Taken together, these data suggest that a decrease in 5-HT uptake occurs at serotonergic nerve terminals in adult BDNF+/− mice. Our *in vitro* experiments combined with neurochemical *in vivo* data indicated that the latter alteration is evident in the ventral hippocampus, but not the frontal cortex and dorsal raphe nucleus of BDNF+/−mice. It seems therefore that 5-HT neurotransmission is regulated by BDNF in a region-dependent manner.

4. Strategy 2: effects of increasing BDNF levels on dialysate 5-HT in the hippocampus of adult wild-type BDNF+/+mice

4.1. Effects of local intra-hippocampal BDNF injection

Intra-hippocampal injection of BDNF (100 ng, but not 20 ng) decreased extracellular 5-HT levels in the hippocampus such as that 5-HT outflow was 60 to 70% of basal levels at time points t_{30} and t_{45}, respectively ($P<0.05$ when compared to baseline: Fig. 4A). Furthermore, in mice continuously perfused with an inhibitor of neurotrophin receptor tyrosine kinase, K252a (10 μM), BDNF no longer reduced extracellular levels of 5-HT (Fig. 4B). These data suggest that BDNF, via its binding to TrkB receptors, decreased extracellular levels of 5-HT in the hippocampus of adult mice.

4.2. Effects of local intra-hippocampal BDNF injection on paroxetine-induced changes in hippocampal extracellular 5-HT levels

In the first part of this experiment, a systemic administration of paroxetine significantly increased dialysate 5-HT levels in the hippocampus from t_{30} to t_{120}, ($P<0.001$ when compared to the respective basal values; Fig. 5A time course effects and 5B AUC values for 5-HT). Then, at t_{75}, i.e., 15 min after intra-hippocampal BDNF injection, dialysate 5-HT levels in the BDNF-treated group were significantly higher than those measured in the control group ($P<0.05$). Thus, BDNF potentiated the effects of a systemic administration of paroxetine on dialysate 5-HT levels in the adult hippocampus in mice.

In these microdialysis experiments, the exact location of the probes was verified according to Bert et al. (2004). Coronal sections of a wild-type mouse brain shows the location of the concentric microdialysis probe (Fig. 5C). The probes were implanted with the following stereotaxic coordinates from Bregma (in mm) in the ventral hippocampus: AP-3.4; L 3.4; V-4.0.

5. Final remarks

The present study, mostly using intracerebral *in vivo* microdialysis, assessed whether a decrease (a constitutive deletion of one copy of the BDNF gene during development) or an increase (a local intra-hippocampal BDNF injection) in BDNF protein levels in the mouse brain can affect hippocampal 5-HT transmission in adulthood. Our *in vivo* approaches demonstrate

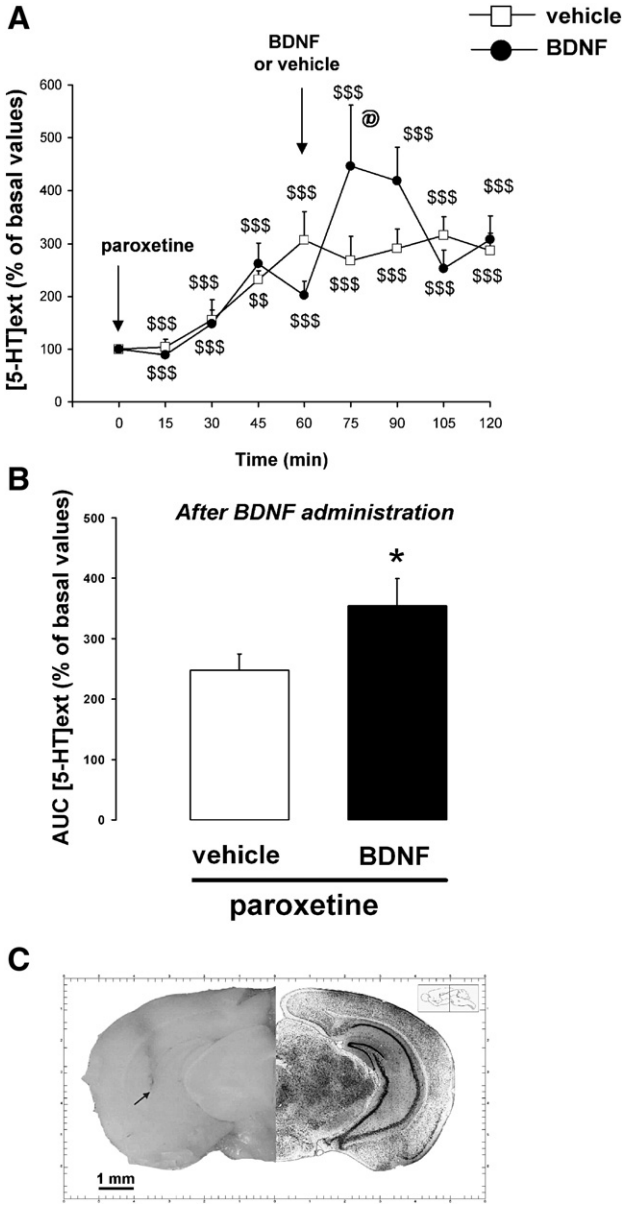

Fig. 5. Effects of intra-hippocampal perfusion of BDNF on paroxetine-induced increases in extracellular 5-HT levels in the ventral hippocampus of freely moving wild-type mice. Basal dialysates 5-HT levels in the vHi of mice treated did not significantly differ between these groups of mice [(in fmol/20 μL) (mean±SEM) 4.15±0.67 (n=7); 3.66±0.5 (n=9) for protein/vehicle and paroxetine/BDNF respectively] (F (1,14)=0.348, P>0.05). A: Time course: Data are means±SEM of dialysate 5-HT expressed as percentages of basal values. Mice received (arrow) either paroxetine (4 mg/kg; i.p.)/vehicle (□) or paroxetine (4 mg/kg; i.p.)/BDNF (100 ng) (■). B: Area under the curve (AUC; mean±SEM) values calculated for the amount of 5-HT outflow in the vHi measured after the perfusion of either vehicle or BDNF 60–120 min (B) post treatment period and expressed as percentages of baseline (7–11 mice per group). *P<0.05; from corresponding vehicle-treated group (Two-way ANOVA, Fisher's PLSD *post hoc* test). i.p. intraperitoneal. @P<0.05 significantly different from paroxetine/vehicle treated group at the corresponding time (Two-way ANOVA, Fisher's PLSD *post hoc* test) §§§P<0.001 from t_0 to t_{120} from baseline value for each group (ANOVA for repeated measures, Fisher's PLSD *post hoc* test). C: Coronal section of a wild-type mouse brain showing the location of the microdialysis probe according to Paxinos and Franklin (2001). The probes were implanted in the ventral hippocampus. The length of the black bar corresponds to 1 mm. The arrow indicates the tip of the microdialysis membrane.

that both strategies led to changes in basal extracellular 5-HT levels in the hippocampus. In both cases, decreasing or increasing BDNF protein levels altered 5-HT uptake, i.e., the 5-HT transporter SERT function in the mouse adult hippocampus.

When BDNF gene expression was reduced (in heterozygous BDNF+/−mice), basal extracellular 5-HT levels increased at serotonergic nerve terminals in the hippocampus. Indeed, as expected, a decrease in hippocampal SERT activity measured in these mutants results in an increased basal extracellular 5-HT level, thus in an increase in 5-HT neurotransmission. In addition, an acute systemic administration of a SSRI, paroxetine, became inactive in the ventral hippocampus and 5-HT uptake *in vitro* (in synaptosomes) as well as *in vivo* (zero net flux) was blunted. These changes were not detected at serotonergic nerve terminal regions such as the frontal cortex and striatum of adult heterozygous BDNF+/−mice (Szapacs e al., 2004). These latter results are not surprising since the striatum is not a brain region involved in adult neurogenesis and changes in BDNF levels following antidepressant drug treatment. The fact that the constitutive decrease in brain BDNF levels alters the effects of paroxetine in the ventral hippocampus, but neither in the frontal cortex nor in the dorsal raphe nucleus, strongly supports the region-specific alteration of the serotonin transporter SERT in mice.

We extended this observation by applying intracerebral *in vivo* microdialysis in the vicinity of cell bodies of 5-HT neurons located in the dorsal raphe nucleus (Guiard et al., 2007). A reduction in SERT function rather than in SERT densities occurred in the adult hippocampus (Guiard et al., 2007). Changes in SERT mRNA expression in the brain stem of these mutant mice cannot account for these changes. Furthermore, they were not associated with a functional desensitization of 5-HT_{1A} autoreceptors in the raphe nuclei since the capacity of a 5-HT1A receptor agonist to decrease either raphe 5-HT neuronal activity or the body temperature was unchanged in BDNF+/−mice compared to their wild-type littermates (Guiard et al., 2007). These results suggest that BDNF is necessary for an appropriate uptake of 5-HT to occur at serotonergic nerve terminals in the hippocampus of adult mice. The effect of BDNF in the presence of paroxetine could be attributed to an increase in hippocampal 5-HT release.

As expected, when BDNF protein levels were increased (by local intra-hippocampal BDNF injection in wild-type mice), basal extracellular 5-HT levels decreased at serotonergic nerve terminals in the hippocampus. These effects are selective and depend on the activation of TrkB receptors since they were blocked by K252a, an inhibitor of neurotrophin receptor tyrosine kinase.

The hypothesis that decreases in Ca^{2+}-dependent release of 5-HT or increases in 5-HT uptake may be responsible for this decrease must be further investigated. Preliminary data obtained in rats (Benmansour et al., Soc. For Neurosci. Atlanta, USA, 2006) suggest that BDNF has neither effect on the affinity of a SSRI for SERT binding sites nor on SERT density in CA3 region of adult hippocampus. In addition, the effects of an acute systemic administration of paroxetine on dialysate 5-HT was potentiated by BDNF injection in the hippocampus. Thus, in this case, SERT the main target of this antidepressant drug is selectively inhibited by this SSRI. BDNF further increased the amount of 5-HT in hippocampal synapses.

Recent reports suggest that neurogenesis is associated with chronic, but not acute, administration of all various types of antidepressant drugs in both the subventricular zone and adult hippocampus. At the present time, increases in various phases of neurogenesis (cell proliferation, migration, differentiation, survival of newly formed neurons and synaptogenesis) were observed in adult hippocampus following chronic treatment with antidepressant drugs such as tranylcypromine, reboxetine, (Malberg et al., 2000); fluoxetine (Malberg et al., 2000; Manev et al., 2001; Santarelli et al., 2003); tricyclic antidepressant drugs, e.g., imipramine, desipramine (Santarelli et al., 2003; Chen et al., 2006a; Holick et al., 2007); the CRF(1) receptor antagonist SSR125543A and the V(1b) receptor antagonist SSR149415 (Alonso et al., 2004); agomelatine (Banasr et al., 2006); MCHR1 receptor antagonist, SNAP 94847 (David et al., 2007).

Similarly, intra-hippocampal BDNF infusion for two weeks increased neurogenesis of granule cells in the dentate gyrus of the hippocampus in adult rats (Scharfman et al., 2005).

There is a great deal of interest in neurotrophin therapy to prevent neurodegenerative diseases as well as to treat mood disorders. However, we need first to investigate growth factors' effects in various animal models of anxiety-depression. Indeed, all the above described studies using SSRIs have been performed in normal animals. In mice subjected to the chronic mild stress (CMS) procedure, a model of depression with predictive validity (Alonso et al., 2004), repeated administration of fluoxetine (10 mg/kg/day i.p. for 28 days) significantly reversed the reduction of cell proliferation produced by CMS. This result suggests that clinically effective antidepressant drugs affect plasticity changes in the hippocampal formation. Furthermore, the large size of neurotrophin and the blood–brain barrier represent major hurdles in the use of peptide therapeutics. Intracerebral microdialysis is a key technique allowing to overcome some of these difficulties.

Taken together, the microdialysis technique gave symmetrical results between the two strategies. BDNF can modulate the amount of 5-HT in synapses either by decreasing (in heterozygous BDNF+/−mice) or increasing (local BDNF injection) the activity of SERT or 5-HT release or both.

According to Altar et al. (1997), neurotrophins can exert different roles. First, their long distance retrograde signalling and participation to the development of the peripheral nervous system involves their retrograde transport from terminals to the cell bodies of neurons. A local action of BDNF in the adult central nervous system (as in the present study after its local injection within the hippocampus of adult mice), involves the anterograde transport, for example, from neuron cell bodies to their terminals, then is released to bind to its post-synaptic receptor on target cells (Altar et al., 1997). Endogenous BDNF is produced by neurons in the peripheral and central nervous systems. BDNF protein and mRNA are distributed throughout the brain, thus suggesting that both retrograde and anterograde transports are probably widespread. We can thus infer that, in our experimental conditions, exogenous BDNF either was taken up by presynaptic non-serotonergic neurons and was released in synapses, or acted directly on post-synaptic serotonergic nerve terminals located in the hippocampus, through its binding to TrkB receptors. The post-synaptic localization of the full-length, active form of these receptors was already demonstrated in adult rat cerebral cortex and hippocampus (Wu et al., 1996).

BDNF is known to enhance synaptic neurotransmission in hippocampal neurons through TrkB receptor activation at excitatory glutamatergic synapses and phosphorylation of post-synaptic ionotropic receptors such as *N*-methyl-D-asparate (NMDA) receptors (Suen et al., 1997). BDNF thus modulates hippocampal long-term potentiation (LTP), a cellular and molecular model of plasticity associated with learning and memory. However, intra-hippocampal injection of BDNF decreased rather than increased clearance rate of 5-HT. How can BDNF decrease hippocampal 5-HT release here? The 5-HT outflow measured with the microdialysis technique is a balance between 5-HT uptake and its release. Taken together, the data obtained with BDNF and co-administration of BDNF with paroxetine suggest that the effects of BDNF on 5-HT uptake predominates over those on 5-HT release, thus leading to a decrease in dialysate 5-HT levels following a single administration of BDNF.

According to monoaminergic hypothesis of depression an increase in 5-HT levels lead to antidepressant-like activity in rodents. Since BDNF modulates 5-HT outflow would BDNF have an antidepressant like activity in rodents? Previous studies found that infusion of BDNF into the brain produced antidepressant-like activity in various animal models of depression (Siuciak et al., 1997). The dose we used has more physiological relevance than a higher dose since the total amount of BDNF in the hippocampus corresponds to a weight of about 150 ng/g wet weight tissue (Szapacs et al., 2004). In another study, intra-hippocampal BDNF injection in rats induced an antidepressant-like effect that was dose-dependent (a dose as low as 0.25 μg of BDNF induced it), was observed 3 days and lasted up to 10 days after its bilateral injection (Shirayama et al., 2002). Whether or not behavioural and neurochemical responses could be associated with TrkB receptor expression in particular hippocampal subfields, needs to be investigated. In this latter study, the diffusion of BDNF from the site of injection was limited (≈0.5 mm) and peak levels of BDNF immunolabeling were observed 2 h after injection to rats. Multiple infusions gave an effect similar to that of a single injection (Shirayama et al., 2002). In the present study, maximal effects of BDNF on dialysate 5-HT was found in the hippocampus 45 min after injection to mice. Since BDNF potentiates the effects of paroxetine on dialysate 5-HT, we are currently investigating whether a co-administration of BDNF and paroxetine lead to antidepressant-like activity in mice.

6. Conclusion

The present data may help better understand the physiopathology of depression and the mechanism of antidepressant efficacy as they link abnormalities of two distinct neurotransmitter systems (i.e. reduced BDNF expression and reduced 5-HT re-uptake). Thus, both SERT and BDNF may be implicated in the mechanism of action of antidepressant drugs. SERT gene presents a polymorphism in its promoter region. Mice carrying one or two methionine allele corresponding to the human methionine BDNF gene polymorphism display anxiety like

behaviour (Chen et al., 2006b). Genetically modified animals help identifying interactions between SERT and BDNF, brain regions and neuronal pathways involved in these interactions: this research area might be fruitful to improve antidepressant treatment and/or to discover new therapeutic targets. Today, it is quite difficult to consider BDNF as a treatment of depressive disorders because of its peptidic structure, BDNF is rapidly degraded by endopeptidases. Small non-peptidic TrkB receptor agonists that cross the blood brain barrier have to be designed.

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