

Chronic treatment with fluoxetine up-regulates cellular BDNF mRNA expression in rat dopaminergic regions

Raffaella Molteni¹, Francesca Calabrese², Francesco Bedogni¹, Enrico Tongiorgi³,
Fabio Fumagalli¹, Giorgio Racagni^{1,2} and Marco Andrea Riva¹

¹ Center of Neuropharmacology, Department of Pharmacological Sciences and Center of Excellence on Neurodegenerative Diseases (CEND), University of Milan, Milan, Italy

² I.R.C.C.S. San Giovanni di Dio-Fatebenefratelli, Brescia, Italy

³ Department of Biology, University of Trieste, Trieste, Italy

Abstract

During the last few years several studies have highlighted the possibility that major depression can be characterized by a general reduction in brain plasticity and an increased vulnerability under challenging situations. Such dysfunction may be the consequence of reduced expression and function of proteins important for neuroplasticity such as brain-derived neurotrophic factor (BDNF). On this basis, by using a sensitive non-radioactive in-situ hybridization, we evaluated the effects of a chronic treatment with fluoxetine on BDNF expression within rat dopaminergic regions. In fact, besides the well-established role of the hippocampus, increasing evidence indicates that other brain regions may be involved in the pathophysiology of depression and consequently be relevant for the therapeutic action of antidepressant drugs. Our results indicate that 3 wk of fluoxetine administration up-regulates BDNF mRNA levels selectively within structures belonging to the meso-cortico-limbic pathway. The expression of the neurotrophin is significantly increased in the ventral tegmental area, prefrontal cortex, and shell region of the nucleus accumbens, whereas no changes were detected in the substantia nigra and striatum. Moreover, in agreement with previous studies, fluoxetine increased BDNF mRNA levels in the hippocampus, an effect that was limited to the cell bodies without any change in its dendritic targeting. These data show that chronic treatment with fluoxetine increases BDNF gene expression not only in limbic areas but also in dopaminergic regions, suggesting that such an effect may contribute to improve the function of the dopaminergic system in depressed subjects.

Received 3 January 2005; Reviewed 24 February 2005; Revised 2 May 2005; Accepted 5 May 2005;
First published online 22 July 2005

Key words: Antidepressants, dopaminergic system, gene expression, in-situ hybridization, neurotrophin.

Introduction

Depression is a complex disease characterized by heterogeneous symptoms. It is widely accepted that its pathophysiology is associated with decreased synaptic concentration of monoamines (Leonard, 2000). However, whereas antidepressant drugs rapidly enhance monoamine concentrations, the therapeutic effects are delayed by several weeks supporting the idea that long-term adaptive changes, involving intraneuronal signal transduction and gene expression modulation,

are required for therapeutic activity. These adaptive changes are thought to normalize the reduction in brain plasticity and enhance the cellular resilience that may be reduced in mood disorders (Duman, 2002; Manji and Lenox, 2000).

Among the molecular elements known to regulate neuronal plasticity, the neurotrophin brain-derived neurotrophic factor (BDNF) plays a crucial role (Manji et al., 2003). Several studies have suggested a role for BDNF as a mediator of the therapeutic action of antidepressants (Hashimoto et al., 2004). According with the time-course of their clinical effects, the expression of BDNF is increased in the hippocampus in response to repeated but not acute treatment with major classes of antidepressants (Coppell et al., 2003; De Foubert et al., 2004; Nibuya et al., 1995, 1996; Russo-Neustadt

Address for correspondence: Professor M. A. Riva, Center of Neuropharmacology, Department of Pharmacological Sciences, Via Balzaretti, 9, 20133 Milan, Italy.
Tel.: +39-0250318334 Fax: +39-0250318278
E-mail: M.Riva@unimi.it

et al., 1999). Moreover, intrahippocampal BDNF infusion is effective in two classical behavioural models of depression, the learned helplessness model and the forced swim test (Shirayama et al., 2002).

In addition to the hippocampus, which has a pivotal role in mood disorders (Sheline et al., 2002), other brain regions may contribute to the disease symptomatology. In fact, hippocampal atrophy, which may be a consequence of enhanced stress exposure, is not always observed in depressed subjects (Mervaala et al., 2000; Shah et al., 1998; Vakili et al., 2000). In addition, human studies have shown blood-flow changes not only in the hippocampal region but also at cortical and subcortical levels (Drevets, 2001; Liotti and Mayberg, 2001) supporting the idea of a 'neural circuitry of depression' in which dopaminergic areas (i.e. ventral tegmental area, substantia nigra, nucleus accumbens) also represent important components (Nestler et al., 2002). Interestingly, these areas are part of the brain reward pathway and any dysfunction at this level may contribute to anhedonia, a loss of pleasure and interest in nearly all activities, which represents an important feature of major depression (Naranjo et al., 2001). An effective approach for studying the role of dopaminergic regions in depression is to measure changes in the expression levels of genes following antidepressant treatment. To this regard, BDNF represents an ideal candidate to evaluate the effects of antidepressants in dopaminergic regions. Moreover, BDNF has been implicated in the maturation and maintenance of midbrain dopaminergic neurons during adulthood (Hyman et al., 1991). Further, as mentioned above, its regulation by antidepressant drugs has previously been shown (Coppell et al., 2003; De Foubert et al., 2004; Nibuya et al., 1995, 1996; Russo-Neustadt et al., 1999). Therefore, it can be hypothesized that neuroplastic changes, so far described in the hippocampus, may also take place in dopaminergic areas, thus contributing to the normalization of these defective functions. On this basis, in the present study, we have investigated the effects produced by chronic administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine on the expression profile of BDNF in dopaminergic pathways that could play a role in some aspects of mood disorders.

Materials and method

Materials

General reagents were purchased from Sigma-Aldrich (Milan, Italy), and molecular biology reagents from Ambion (Austin, TX, USA), New England Biolabs

(Beverly, MA, USA) and Promega Italy (Milan, Italy). Fluoxetine was obtained from Eli Lilly (Sesto Fiorentino, Italy).

Animals and drug treatment

Adult male Sprague–Dawley rats (225–250 g, Charles River, Calco, Italy) were housed on a 12 h light/dark cycle, with free access to food and water for 1 wk and then randomly assigned to receive daily injections (between 09:00 and 10:00 hours) of fluoxetine (10 mg/kg i.p.) or saline for 3 wk ($n=12$ in each experimental group). Twenty-four hours after the last injection, 16 animals were sacrificed by decapitation ($n=8$ /group) and the following brain regions were rapidly dissected from 2-mm-thick slices hippocampus: [HIP, defined as CA1, CA2, CA3, and DG subregions corresponding to plate 31 (plates refer to the atlas of Paxinos and Watson 1996)], prefrontal cortex (PFC, defined as Cg1, Cg3, and IL subregions corresponding to plates 6–9), ventral tegmental area (VTA, defined as RMC and PBP subregions corresponding to plate 40), striatum (STR, defined as CPu region corresponding to plate 12), and nucleus accumbens (NAc, defined as AcbH and AcbC subregions corresponding to plate 10). The specimens were frozen on dry ice and stored at -70°C for ELISA immunoassay. A separate cohort of rats ($n=4$ /group) were killed by 4% paraformaldehyde transcardial perfusion under deep anaesthesia and their brains were used for in-situ hybridization as described below.

All efforts were made to minimize animal suffering and to reduce the number of animals employed in the study. All experiments were performed in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (DHEW Publications, NIH 80-23), with the EC guidelines (EC Council Directive 86/609) and with the Italian legislation on animal experimentation (Decreto Legislativo 116/92).

In-situ hybridization

Rats were transcardially perfused with 4% paraformaldehyde under ketamine anaesthesia, and their brains were removed and kept in 4% paraformaldehyde/20% sucrose at 4°C for at least 3 d before sectioning. Free floating, $40\ \mu\text{M}$ coronal sections were obtained with a freezing microtome (Leica Histo 2000, Nussloch, Germany) and then post-fixed in paraformaldehyde 4%/PBS and stored in this medium at 4°C until use. Sections containing the dopaminergic areas under investigations (Paxinos and Watson, 1996) underwent in-situ hybridization performed as previously described (Tongiorgi et al., 1998). A 480-bp

antisense probe complementary to the rat BDNF coding sequence was prepared from a cDNA (*XbaI-PstI*) insert cloned in a pGEM3Z plasmid. After linearization with *EcoRI*, digoxigenin-labelled riboprobe was synthesized with a Sp6 polymerase (Sp6-T7 DIG-RNA labelling kit, La Roche, Mannheim, Germany, according to the manufacturer's instructions). The specificity of the riboprobe used was demonstrated previously (Capsoni et al., 1999; Tongiorgi et al., 1997). Prehybridization was carried out at 55 °C in 50% deionized formamide, 20 mM Tris-HCl, 1 mM EDTA, 300 mM NaCl, 100 mM dithiothreitol (DDT), 1× Denhardt's solution, 0.5 mg/ml ssDNA, 0.5 mg/ml polyadenylic acid. In-situ hybridization was performed overnight at 55 °C in the prehybridization mix, to which 10% dextran sulphate and the riboprobe (50–100 ng/ml) were added. High-stringency washes were performed in 0.1× SSC/0.1% Tween-20 at 60 °C. Sections hybridized with digoxigenin-labelled riboprobe were incubated overnight at 4 °C with anti-DIG Fab fragments coupled to alkaline phosphatase diluted 1:500 in 10% fetal calf serum. Sections were then washed four times in PBS, and reacted with 4-nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate in 100 mM Tris-HCl, 50 mM MgCl₂, 100 mM NaCl, and 1 mM levamisol. To obtain reproducible and comparable results and to avoid saturation of the reaction, alkaline phosphatase development was always performed for 4 h at room temperature. Finally, sections were mounted on gelatin-coated slides, dried for 30 min at 55 °C, rinsed in methanol for 30 s, in xylene for 3 min, and then coverslips were mounted with DPX mountant for histology.

Protein isolation and measurement

Tissues from various brain structures were homogenized in a glass-glass potter in a cold lysis buffer (pH 7.4) (0.32 M sucrose, 1 mM Hepes solution, 0.1 mM EGTA, 0.1 mM phenylmethylsulphonyl fluoride) with a complete set of protease inhibitors and a phosphatase inhibitor cocktail. Homogenates were centrifuged to remove insoluble material (10 000 g for 20 min at 4 °C) and total protein concentration was determined according to the Bio-Rad protein assay (Bio-Rad, Milan, Italy) procedure.

BDNF protein was quantified using an enzyme-linked immunosorbent assay (ELISA) kit (BDNF Emax ImmunoAssay System kit; Promega Inc., Milan, Italy) as per manufacturer's protocol. Briefly, Nunc MaxiSorp 96-well plates were coated with 0.1 ml of a monoclonal antibody against BDNF in a buffer (pH 9.7) containing 0.025 M sodium bicarbonate and

0.025 M sodium carbonate for 16 h at 4 °C. After washing in TBS-T [20 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.05% Tween-20], the wells were incubated with 0.2 ml of a blocking buffer at room temperature for 1 h and then washed in TBS-T again. Samples, six serial dilutions of a BDNF standard (500 pg/ml), and a blank (no BDNF) were added in duplicate into separate wells. Plates were incubated for 2 h at room temperature and washed five times in TBS-T. A polyclonal antibody against BDNF (1:500 dilution) was added into each well and plates were incubated for 2 h at room temperature. After five washes in TBS-T, 0.1 ml of a secondary anti-IgY antibody with a horseradish peroxidase conjugate was added to each well and plates were incubated for 1 h at room temperature. Wells were washed five times with TBS-T. A hydrogen peroxidase solution with a peroxidase substrate was added and incubated for 10 min at room temperature. Reactions were stopped with 1 M chloridric acid and absorbance at 450 nm was measured using an automated microplate reader. Standard curves were plotted for each plate. Duplicates were averaged and values were corrected for total amount of protein in the sample.

Densitometric and statistical analyses

For the densitometric analysis of the in-situ hybridization staining levels images were acquired through a Nikon EMX1200 video-camera mounted on a Nikon E800 microscope (Nikon Italia s.r.l., Florence, Italy) and analysed with the software Image Pro Plus[®] 5.0 for Windows (Media Cybernetics, Silver Spring, MD, USA). Illumination was adjusted to obtain optimal staining signal over the dendritic fields and the cell somas, and then was kept constant throughout all experiments. Staining levels were expressed as grey levels ranging from 0 (black) to 255 (white). Data obtained from each animal were normalized on the average grey level measured within the white matter (corpus callosum). For the mRNA targeting analysis each section was positioned to align the pyramidal or the granular cell layer parallel to one border of the field visualized by the camera and densitometric analysis was carried out at the constant distances from the beginning of the dendrites as previously described (Tongiorgi et al., 2004). Three independent analyses were performed on each animal used. For each region, the densitometry was performed on three sections per animal (animals per group $n=4$; total sections per group $n=12$). The intensity values of the three sections were averaged to give the mean value of each single animal and used to calculate the mean intensity value

for the two experimental groups (fluoxetine vs. control), their variances, and standard errors. The mean value of each animal, obtained from the three independent analyses mentioned, was then divided for the mean of the control group and the intensities were therefore expressed as a percentage of the control group average (=100%).

Statistical evaluation of the changes in BDNF levels were performed using Student's *t* test and the differences were considered significant when $p < 0.05$. In the bar graph, the mean value of the control group (saline-treated animals) for each brain region is expressed as 100 and the data of animals injected with fluoxetine are shown as percentage of saline-treated rats. The values represent the mean \pm standard error of the mean (S.E.M.) of four (in-situ hybridization) or eight (ELISA) independent determinations.

Results

Several studies indicate that, in addition to the hippocampus, other brain regions may mediate symptoms of depression and could, therefore, represent putative targets for pharmacological treatment (Mayberg, 2002; Nestler et al., 2002). A sensitive non-radioactive in-situ hybridization was then used to investigate the expression and possible differences in the localization of BDNF mRNA levels in different brain structures of rats chronically treated with the antidepressant fluoxetine. The specificity of the riboprobe used was previously demonstrated (Capsoni et al., 1999; Tongiorgi et al., 1997).

Figure 1 illustrates the distribution of BDNF mRNA in the hippocampal formation of saline- or fluoxetine-treated rats. As shown in Figure 1a, the localization of BDNF mRNA in the hippocampus of saline-treated animals was similar to that reported previously (Schmidt-Kastner et al., 1996) whereas, as documented by other authors (Nibuya et al., 1995, 1996), chronic antidepressant treatment enhanced the expression of the neurotrophin in selected hippocampal subfields (Figure 1b,d,f,h). The quantitative densitometric analysis (Figure 2) showed that chronic administration of fluoxetine significantly increased BDNF mRNA levels in the soma of dentate gyrus (DG) granule cells (+15%, $p = 0.04$) and of the CA1 neurons (+19%, $p = 0.02$) but not in neurons of the CA3 region (+4%, $p = 0.54$) (Figure 1b). The changes produced by fluoxetine were limited to the soma of the neurons, whereas no staining was detected in the proximal or distal part of dendrites (Figure 2), suggesting that prolonged antidepressant treatment, unlike more intense stimuli such as kindling, or pilocarpine- and kainate-induced

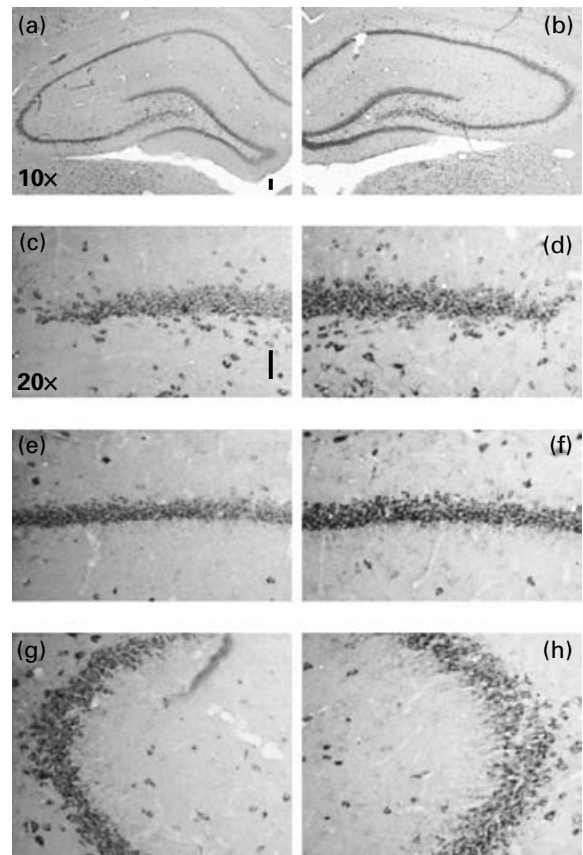


Figure 1. Effect of chronic treatment with fluoxetine on BDNF gene expression in the rat hippocampal formation. Coronal sections of dorsal hippocampus (plate 31 of Paxinos and Watson, 1996) from both saline-treated (a, c, e, g) and 3-wk fluoxetine-treated (b, d, f, h) rats representative of individual animals ($n = 4$ animals/group) were hybridized with digoxigenin-labelled riboprobes. In fluoxetine-treated rats higher levels of BDNF staining were found in the dentate gyrus (DG) granule cell bodies (d) and in the CA1 pyramidal cells (f), whereas no induction in the transcription was found in the CA3 region (h). Although there was increased staining in the cell bodies of DG and CA1 (d, f), we found no BDNF signal along the dendrites of both the subregions and within the CA3 (h).

seizures (Tongiorgi et al., 2004), did not induce dendritic targeting of the mRNA for this neurotrophin.

In order to evaluate the effects of the chronic treatment with fluoxetine on dopaminergic circuitry, we analysed BDNF mRNA expression in the VTA (Figure 3c,d) and substantia nigra (SN; Figure 3e, f), containing dopaminergic cell bodies, as well as in the PFC and NAc, which represent two targets of efferent dopaminergic fibres and may contribute to cognitive and anhedonic features of the disease.

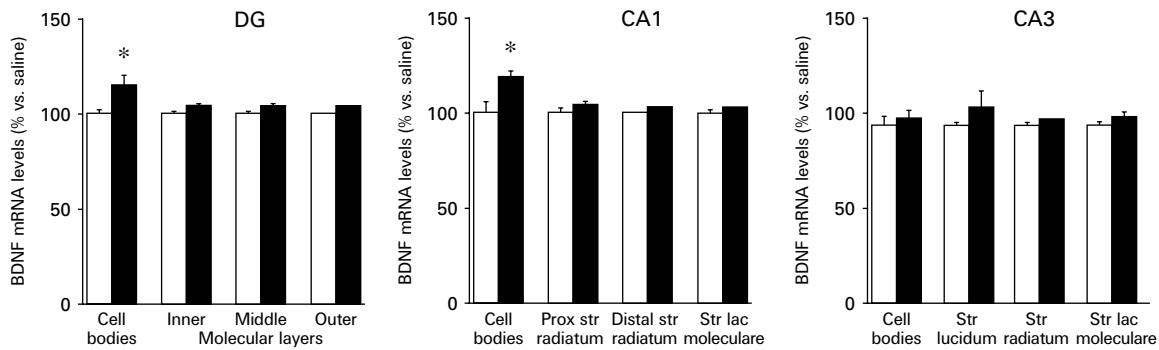


Figure 2. Quantitative densitometric analysis of the effect of chronic treatment with fluoxetine on hippocampal BDNF mRNA levels in the cell bodies and along the dendrites performed as described in the Materials and methods section. The results are expressed as percentage of control levels (100% for saline-treated animals) and represent the mean \pm S.E.M. of four independent determinations (* $p < 0.05$ unpaired two-tailed Student's *t* test). ■, Fluoxetine; □, saline.

As shown in Figure 3a,c,e, many BDNF-labelled cells were observed in the VTA and the SN of control animals. After fluoxetine treatment, the staining appeared to increase in both structures (Figure 3b,d,f) but the results of the densitometric analysis (summarized in Figure 4) indicated that this effect was significant only in the VTA (+22%, $p = 0.04$), whereas no significant changes were found in different subfields of the SN (pars compacta, pars reticulata, pars laterale).

In the PFC (Figure 5) fluoxetine elicited a significant elevation of BDNF mRNA levels in the soma of pyramidal cells, an effect confirmed by the quantitative densitometric analysis (Figure 7a, +15%, $p = 0.003$). In the NAc (Figure 6), the expression of BDNF mRNA was low in control animals (Figure 6a, c), and slightly increased following chronic fluoxetine treatment (Figure 6b,d). This effect was statistically significant only within the shell (Figure 7b, +17%, $p = 0.04$) but not the core (Figure 7b, +15%, $p = 0.09$).

The analysis of BDNF protein levels (Figure 8) revealed only a partial match to the changes observed at mRNA levels. In fact, whereas the up-regulation of BDNF gene expression was detected in several brain regions including hippocampus, VTA, PFC and NAc, fluoxetine significantly increased BDNF protein only in the PFC (+22%, $p = 0.003$). Conversely, only a trend to an increase was found in the VTA (+10%, $p = 0.07$) and no differences were detected in the hippocampus, NAc, and STR of fluoxetine-treated animals in comparison with saline-injected rats.

Discussion

The so-called 'neurotrophic hypothesis of depression' suggests that decreased levels of the neurotrophin BDNF may contribute to the development of mood

disorders and that effective treatment may eventually be achieved by elevating the expression of this neurotrophin. Three main evidences strongly support this theory. First, BDNF expression is decreased in response to stress, a well-recognized precipitating event of depression (Alfonso et al., 2004; Roceri et al., 2002, 2004; Smith et al., 1995a,b). Second, chronic antidepressant treatment increases BDNF levels in limbic regions (Coppell et al., 2003; De Foubert et al., 2004; Nibuya et al., 1995, 1996; Russo-Neustadt et al., 1999). Finally, infusion of BDNF into the DG of the hippocampus produces antidepressant effects in two animal models of depression (Shirayama et al., 2002). To date, the majority of these reports have been focused on the hippocampus, according to its well-established role in depression (Sheline et al., 2002). Our results demonstrate that the expression of BDNF is modulated not only in the hippocampus but also in selected dopaminergic regions in response to chronic treatment with the SSRI fluoxetine. The changes of neurotrophin expression appear to be restricted to the meso-limbic and meso-cortical pathways: enhanced BDNF levels are indeed observed in VTA, PFC and the shell of the NAc, but not in SN or STR. Our results are consistent with a recent paper reporting an increase of BDNF gene expression in the rat VTA/SN region after chronic treatment with the tricyclic antidepressant imipramine (Van Hooissen et al., 2003). Using the non-radioactive variant of the in-situ hybridization technique, we were able to discriminate the effects in VTA with respect to the SN. In addition, the high spatial resolution obtained with the non-radioactive in-situ technique, allowed us to detect differences in BDNF mRNA levels between the shell and the core within the NAc, in spite of the low endogenous levels of expression of BDNF in this area.

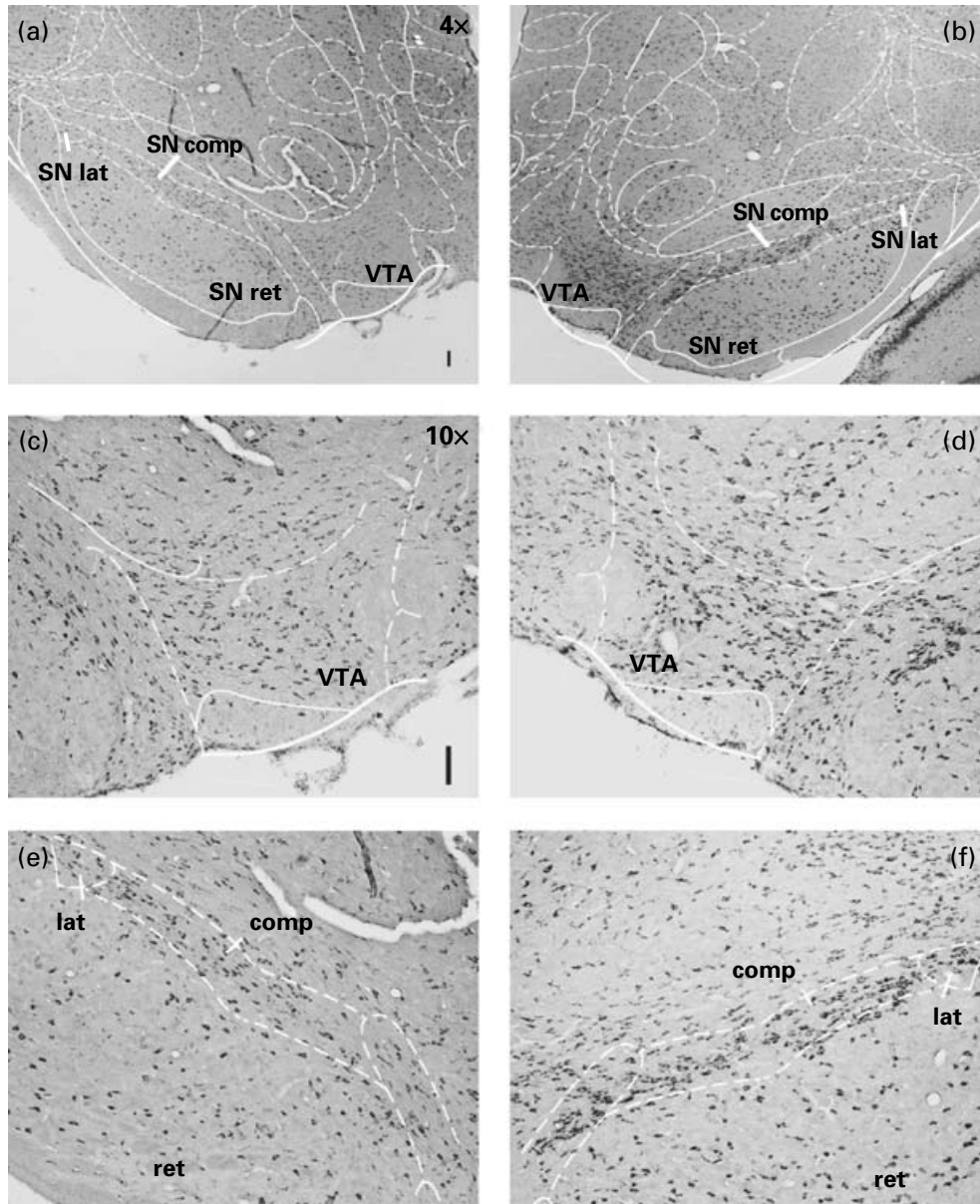


Figure 3. Effect of chronic treatment with fluoxetine on BDNF gene expression in the rat midbrain. Coronal sections of midbrain region (plate 41 of Paxinos and Watson, 1996) of saline-injected (a) and fluoxetine-injected rats (b) were hybridized as described in the Materials and methods section to detect BDNF mRNA levels. BDNF staining was increased in the VTA of fluoxetine-treated (d) in comparison with saline-treated animals (c) whereas, within the SN (f), the signal of the neurotrophin was not different from control levels (e). SNcomp, SN pars compacta; SNret, SN pars reticulata; SNlat, SN pars laterale.

The selective increase of the neurotrophin following chronic administration of fluoxetine may have functional consequences related to the anatomical localization. The meso-limbic and meso-cortical dopaminergic pathways are implicated in the physiology of reward and motivation and in the pathophysiology of drug abuse (Spanagel and Weiss, 1999), but increasing evidence supports their involvement also in

mood disorders (Dailly et al., 2004). Clinical as well as preclinical studies suggest a relationship between dopamine transmission and depression. For example, an up-regulation of D_2 receptor density was observed in the basal ganglia/cerebellum of depressed patients in comparison with healthy subjects (D'haenen and Bossuyt, 1994). It has also been shown that the meso-cortico-limbic dopaminergic pathway has a permissive

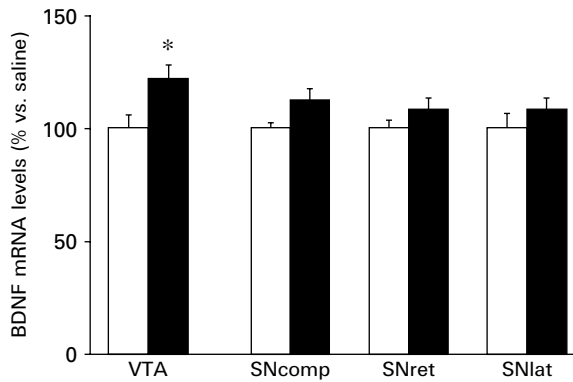


Figure 4. Quantitative densitometric analysis of the effects of chronic treatment with fluoxetine on BDNF gene expression in the VTA and SN pars compacta (SNcomp), pars reticulata (SNret), pars laterale (SNlat). The results, expressed as percentage of control levels (100% for saline-treated rats), represent the mean \pm s.e.m. of four independent determinations (* $p < 0.05$ unpaired two-tailed Student's t test). ■, Fluoxetine; □, saline.

role in the effect of the antidepressant desipramine in the forced swim test (Cervo et al., 1990). Moreover, based on the role of this pathway in motivation and reward, it is possible to hypothesize its involvement in the loss of interest or pleasure (anhedonia), which represents an important feature of depression. Since chronic treatment with fluoxetine reverses the anhedonic effect caused by chronic mild stress (D'Aquila et al., 1997; Muscat et al., 1992), we speculate that the changes in BDNF expression could be relevant for the antidepressant effect of the SSRI in these regions.

A large body of evidence establishes a role for BDNF in dopaminergic neurons. BDNF co-localizes with tyrosine hydroxylase in midbrain dopaminergic cell bodies (Seroogy et al., 1994) suggesting its involvement in the synaptic function of these neurons (Hyman and Malenka, 2001). Infusion of this neurotrophin into the SN, VTA, and NAc increases dopamine utilization and enhances locomotor activity induced by psychostimulant drugs (Pierce and Bari, 2001). Moreover, recent data show that the neurotrophin controls the expression of the D_3 receptor gene (Sokoloff et al., 2002), a major dopamine receptor in the NAc (Sokoloff et al., 1990). It is interesting to note that the D_3 agonist pramipexole, developed as an anti-parkinsonian drug, possesses clinical efficacy in the treatment of unipolar as well as bipolar depression (Corrigan et al., 2000; Zarate et al., 2004).

Our results clearly indicate that chronic treatment with fluoxetine increases BDNF gene expression not only in limbic areas but also in dopaminergic regions,

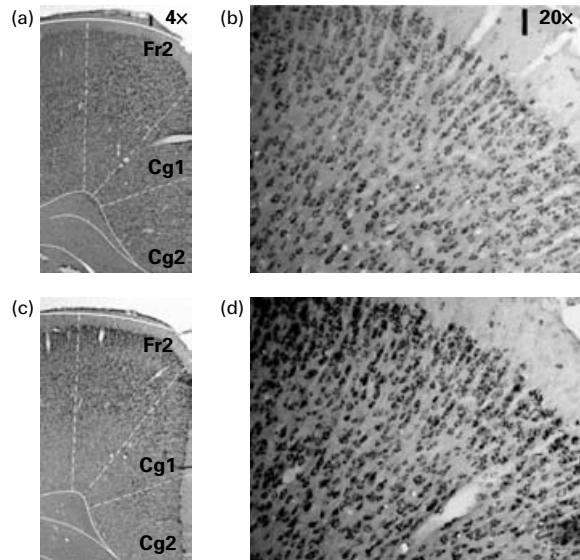


Figure 5. Effect of chronic treatment with fluoxetine on BDNF gene expression in the rat prefrontal cortex. As shown by representative coronal sections (plate 15 of Paxinos and Watson, 1996), the intensity of BDNF signal was higher after fluoxetine administration (c, d) in comparison with control animals (a, b).

suggesting that, also in these structures, the therapeutic effect of the antidepressant may be – at least in part – due to an improvement of neuronal plasticity through the induction of the neurotrophin.

The antidepressant role of BDNF within dopaminergic regions is still controversial. For example, BDNF infusion in the posterior midbrain produces antidepressant activity in two animal models of depression: the learned helplessness model and the forced swim test (Siuciak et al., 1997). On the contrary, two opposite manipulations of BDNF signalling pathway in the VTA–NAc circuitry, i.e. its activation via BDNF infusion in the VTA and its inhibition via infusion of a virus expressing the truncated form of the BDNF receptor, are suggestive of a pro-depressive activity of the neurotrophin in these regions (Eisch et al., 2003). It is extremely difficult to compare the effects of chronic infusion of exogenous BDNF, which may induce specific structural alterations within the areas examined, with a 'more physiological' modulation of endogenous BDNF levels. Nevertheless, we clearly demonstrate that chronic fluoxetine treatment regulates endogenous BDNF levels by increasing its expression within the meso-cortico-limbic dopaminergic pathway.

In our study, the PFC was the only region in which elevated protein levels, measured by the ELISA test,

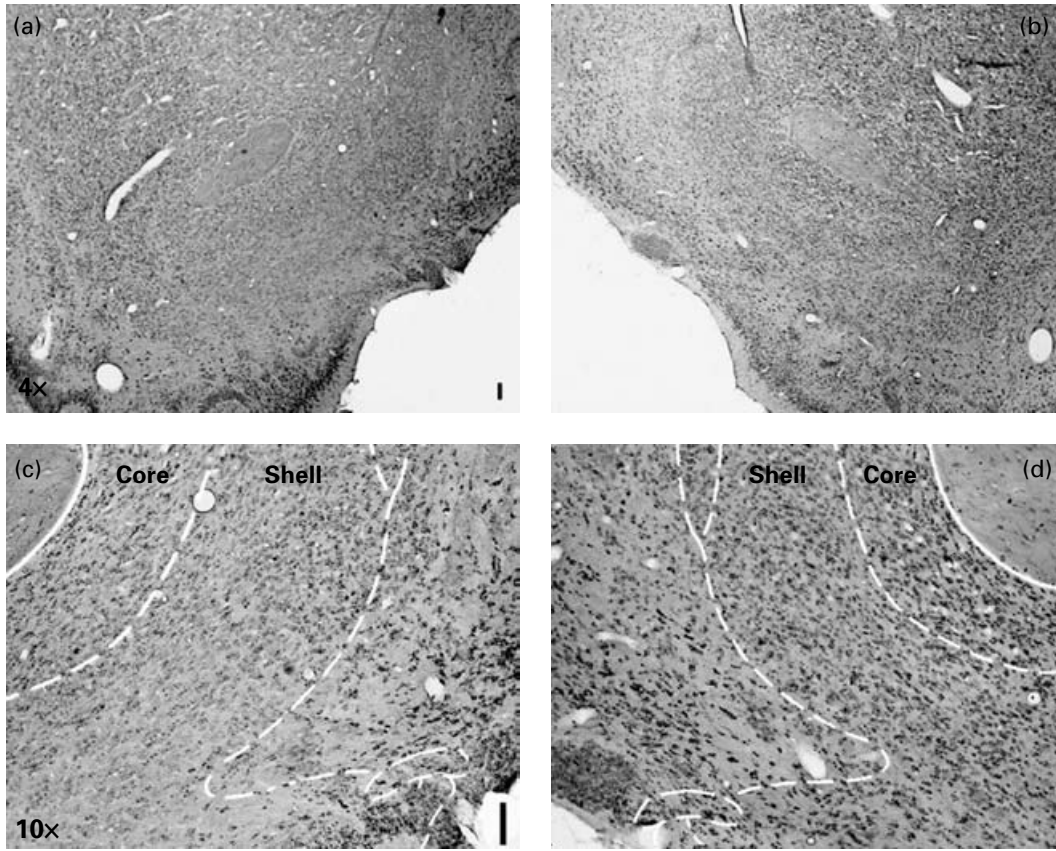


Figure 6. Effect of chronic treatment with fluoxetine on BDNF gene expression in the nucleus accumbens, as shown by representative coronal brain sections (plate 14 of Paxinos and Watson, 1996). Higher BDNF staining was detected within the nucleus accumbens of fluoxetine-injected (b, d) with respect to saline-injected (a, c) rats.

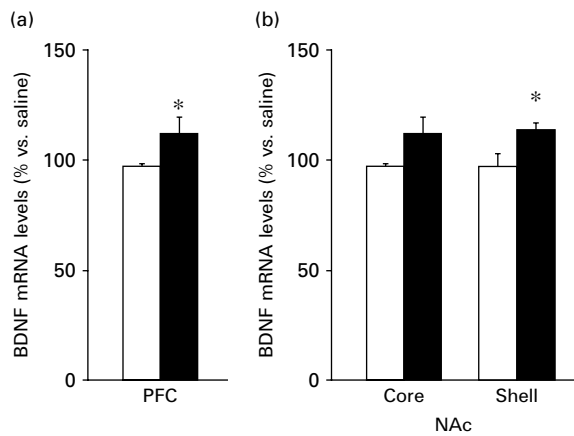


Figure 7. Quantitative densitometric analysis of the effect of chronic treatment with fluoxetine in the prefrontal cortex (PFC) (a) and nucleus accumbens (NAc) (b). The results are expressed as percentage of control levels (100% for saline-treated rats) and represent the mean \pm S.E.M. of four independent determinations (* $p < 0.05$ unpaired two-tailed Student's t test).

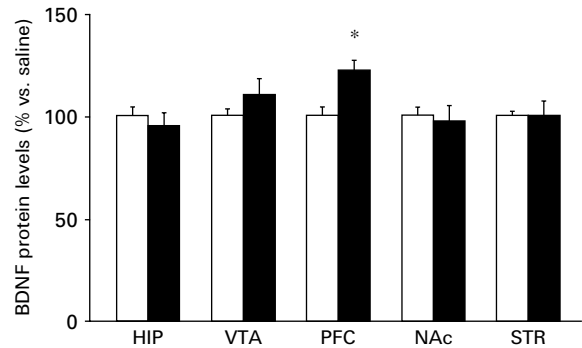


Figure 8. Effect of chronic treatment with fluoxetine on BDNF protein levels measured by ELISA test. BDNF protein levels were analysed in the following brain regions: hippocampus (HIP), ventral tegmental area (VTA), prefrontal cortex (PFC), nucleus accumbens (NAc), and striatum (STR). Data are expressed as percentage of control levels (100% for saline-treated rats) and represent the mean \pm S.E.M. of eight independent determinations (* $p < 0.05$ unpaired two-tailed Student's t test). ■, Fluoxetine; □, saline.

paralleled the up-regulation of BDNF gene expression, an increase that might contribute to the normalization of cognitive deficits often associated with psychiatric disorders (Fossati et al., 2004). Several reasons might be considered to explain the mismatch observed in the other structures analysed. The mRNA analysis was carried out using a high-resolution non-radioactive in-situ hybridization that allowed us to detect small but significant changes of the neurotrophin in specific anatomical structures. It is possible that when changes occur in specific subfields, cell populations, or sub-cellular compartments, the effects could be diluted when evaluated in a whole structure homogenate, as we did for the protein analysis performed by the ELISA method. Therefore, we cannot rule out the possibility that immunohistochemical analysis of BDNF may reveal subtle changes in its protein levels within dopaminergic regions, where mRNA levels are up-regulated. In addition, it is important to consider that new protein could derive not only from new transcription but also from translation of existing mRNA. On this basis, we hypothesize that the elevated BDNF mRNA levels observed in specific subregions of the hippocampus and NAc in response to fluoxetine might represent some kind of 'pool' ready to be translated in case of demand.

Previous studies have reported a mismatch between baseline BDNF mRNA and protein levels in the visual cortex (Tropea et al., 2001) and hippocampus (Conner et al., 1997). Moreover, the lack of effect of chronic fluoxetine on BDNF protein we observed in the hippocampus is in agreement with previous reports (Altar et al., 2003; De Foubert et al., 2004).

In agreement with previous studies, our data show that chronic antidepressant treatment increases BDNF mRNA levels in the hippocampus (Nibuya et al., 1995; Russo-Neustadt et al., 1999). In particular, the expression of the neurotrophin was significantly elevated in the DG and in the CA1 region of the hippocampal formation. Additionally, our results indicate that no BDNF mRNA staining was detected within dendrites suggesting that chronic treatment with fluoxetine was not able to induce dendritic accumulation of BDNF mRNA. Interestingly, in analogy with the present study, electroconvulsive seizures (ECS), a well-known antidepressant treatment, failed to induce accumulation of BDNF mRNA in the distal dendritic compartment (Tongiorgi et al., 2004). A recent study clearly demonstrates that the rat BDNF transcript containing exon IV can be targeted to the distal dendrites while the mRNA isoform bearing exon III is restricted within the soma even after strong stimulation (Pattabiraman et al., 2005). Taken together

these data strongly support the view that fluoxetine may act selectively by increasing the expression of BDNF isoforms that are restricted to the cell soma. This model predicts that chronic fluoxetine treatment should also be associated with an increase in BDNF protein expression within the soma.

In summary, our data provide a new piece of information with regard to the ability of the antidepressant fluoxetine to regulate BDNF expression following chronic administration. Although additional studies are required to further investigate the significance of BDNF increase within the soma of dopaminergic regions, the ability of fluoxetine to up-regulate the neurotrophin in these structures may, at least in part, explain the amelioration of symptoms associated with a dysfunction of the dopaminergic system.

Acknowledgements

This research has been in part supported by funding to M.A.R. from the Italian Ministry of University and Research, the Health Ministry, Ricerca Finalizzata, and by an unrestricted grant from Eli Lilly Italia.

Statement of Interest

None.

References

- Alfonso J, Pollevick GD, Van Der Hart MG, Flugge G, Fuchs E, Frasch AC (2004). Identification of genes regulated by chronic psychosocial stress and antidepressant treatment in the hippocampus. *European Journal of Neuroscience* 19, 659–666.
- Altar CA, Whitehead RE, Chen R, Wortwein G, Madsen TM (2003). Effects of electroconvulsive seizures and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. *Biological Psychiatry* 54, 703–709.
- Capsoni S, Tongiorgi E, Cattaneo A, Domenici L (1999). Dark rearing blocks the developmental down-regulation of BDNF mRNA expression in layers IV and V of the rat visual cortex. *Neuroscience* 88, 393–403.
- Cervo L, Grignaschi G, Samanin R (1990). The role of the mesolimbic dopaminergic system in the desipramine effect in the forced swimming test. *European Journal of Pharmacology* 178, 129–133.
- Conner JM, Lauterborn JC, Yan Q, Galli CM, Varon S (1997). Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *Journal of Neuroscience* 17, 2295–2313.
- Coppell AL, Pei Q, Zetterstrom TS (2003). Bi-phasic change in BDNF gene expression following antidepressant drug treatment. *Neuropharmacology* 44, 903–910.

- Corrigan MH, Denahan AQ, Wright CE, Ragual RJ, Evans DL** (2000). Comparison of pramipexole, fluoxetine, and placebo in patients with major depression. *Depression and Anxiety* 11, 58–65.
- Dailly E, Chenu F, Renard CE, Bourin M** (2004). Dopamine, depression and antidepressants. *Fundamental and Clinical Pharmacology* 18, 601–617.
- D'Aquila P, Monleon S, Borsini F, Brain P, Willner P** (1997). Anti-anhedonic actions of the novel serotonergic agent flibanserin, a potential rapidly-acting antidepressant. *European Journal of Pharmacology* 340, 121–32.
- De Foubert G, Carney SL, Robinson CS, Destexhe EJ, Tomlinson R, Hicks CA, Murray TK, Gaillard JP, Deville C, Xhenseval V, Thomas CE, O'Neill MJ, Zetterstrom TS** (2004). Fluoxetine-induced change in rat brain expression of brain-derived neurotrophic factor varies depending on length of treatment. *Neuroscience* 128, 597–604.
- D'haenen HA, Bossuyt A** (1994). Dopamine D2 receptors in depression measured with single photon emission computed tomography. *Biological Psychiatry* 35, 128–132.
- Drevets WC** (2001). Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. *Current Opinion in Neurobiology* 11, 240–249.
- Duman RS** (2002). Pathophysiology of depression: the concept of synaptic plasticity. *European Psychiatry* 17, 306–310.
- Eisch AJ, Bolanos CA, de Wit J, Simonak RD, Pudiak CM, Barrot M, Verhaagen J, Nestler EJ** (2003). Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. *Biological Psychiatry* 54, 994–1005.
- Fossati P, Radtchenko A, Boyer P** (2004). Neuroplasticity: from MRI to depressive symptoms. *European Journal of Neuropsychopharmacology* 14, S503–510.
- Hashimoto K, Shimizu E, Iyo M** (2004). Critical role of brain-derived neurotrophic factor in mood disorders. *Brain Research Review* 45, 104–114.
- Hyman C, Hofer M, Barde YA, Juhasz M, Yancopoulos GD, Squinto SP, Lindsay RM** (1991). BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* 350, 230–232.
- Hyman SE, Malenka RC** (2001). Addiction and the brain: the neurobiology of compulsion and its persistence. *Nature Review in Neuroscience* 2, 695–703.
- Leonard BE** (2000). Evidence for a biochemical lesion in depression. *Journal of Clinical Psychiatry* 61 (Suppl. 6), 12–17.
- Liotti M, Mayberg HS** (2001). The role of functional neuroimaging in the neuropsychology of depression. *Journal of Clinical Experimental Neuropsychology* 23, 121–136.
- Manji HK, Lenox RH** (2000). Signaling: cellular insights into the pathophysiology of bipolar disorder. *Biological Psychiatry* 48, 518–530.
- Manji HK, Quiroz JA, Sporn J, Payne JL, Denicoff KA, Gray N, Zarate Jr. CA, Charney DS** (2003). Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. *Biological Psychiatry* 53, 707–742.
- Mayberg HS** (2002). Modulating limbic-cortical circuits in depression: targets of antidepressant treatments. *Seminars in Clinical Neuropsychiatry* 7, 255–268.
- Mervaala E, Fohr J, Kononen M, Valkonen-Korhonen M, Vainio P, Partanen K, Partanen J, Tiitonen J, Viinamaki H, Karjalainen AK, Lehtonen J** (2000). Quantitative MRI of the hippocampus and amygdala in severe depression. *Psychological Medicine* 30, 117–125.
- Muscat R, Papp M, Willner P** (1992). Reversal of stress-induced anhedonia by the atypical antidepressants, fluoxetine and maprotiline. *Psychopharmacology* 109, 433–438.
- Naranjo CA, Tremblay LK, Busto UE** (2001). The role of the brain reward system in depression. *Progress in Neuro-psychopharmacology and Biological Psychiatry* 25, 781–823.
- Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM** (2002). Neurobiology of depression. *Neuron* 34, 13–25.
- Nibuya M, Morinobu S, Duman RS** (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *Journal of Neuroscience* 15, 7539–7547.
- Nibuya M, Nestler EJ, Duman RS** (1996). Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *Journal of Neuroscience* 16, 2365–2372.
- Pattabiraman PP, Tropea D, Chiaruttini C, Tongiorgi E, Cattaneo A, Domenici L** (2005). Neuronal activity regulates the developmental expression and subcellular localization of cortical BDNF mRNA isoforms in vivo. *Molecular and Cellular Neuroscience* 28, 556–570.
- Paxinos G, Watson C** (1996). *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press.
- Pierce RC, Bari AA** (2001). The role of neurotrophic factors in psychostimulant-induced behavioral and neuronal plasticity. *Annual Review of Neuroscience* 12, 95–110.
- Roceri M, Cirulli F, Pessina C, Peretto P, Racagni G, Riva MA** (2004). Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. *Biological Psychiatry* 55, 708–714.
- Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA** (2002). Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. *Molecular Psychiatry* 7, 609–616.
- Russo-Neustadt A, Beard RC, Cotman CW** (1999). Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. *Neuropsychopharmacology* 21, 679–682.
- Schmidt-Kastner R, Wetmore C, Olson L** (1996). Comparative study of brain-derived neurotrophic factor messenger RNA and protein at the cellular level suggests multiple roles in hippocampus, striatum and cortex. *Neuroscience* 74, 161–183.

- Seroogy KB, Lundgren KH, Tran TM, Guthrie KM, Isackson PJ, Gall CM** (1994). Dopaminergic neurons in rat ventral midbrain express brain-derived neurotrophic factor and neurotrophin-3 mRNAs. *Journal of Comparative Neurology* 342, 321–334.
- Shah PJ, Ebmeier KP, Glabus MF, Goodwin GM** (1998). Cortical grey matter reductions associated with treatment-resistant chronic unipolar depression. Controlled magnetic resonance imaging study. *The British Journal of Psychiatry* 172, 527–532.
- Sheline YI, Mittler BL, Mintun MA** (2002). The hippocampus and depression. *European Psychiatry* 17, 300–305.
- Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS** (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *Journal of Neuroscience* 22, 3251–3261.
- Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM** (1997). Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacology, Biochemistry and Behavior* 56, 131–137.
- Smith MA, Makino S, Kvetnansky R, Post RM** (1995a). Effects of stress on neurotrophic factor expression in the rat brain. *Annals of the New York Academy of Science* 771, 234–239.
- Smith MA, Makino S, Kvetnansky R, Post RM** (1995b). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *Journal of Neuroscience* 15, 1768–1777.
- Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC** (1990). Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature* 347, 146–151.
- Sokoloff P, Guillin O, Diaz J, Carroll P, Griffon N** (2002). Brain-derived neurotrophic factor controls dopamine D3 receptor expression: implications for neurodevelopmental psychiatric disorders. *Neurotoxicity Research* 4, 671–678.
- Spanagel R, Weiss F** (1999). The dopamine hypothesis of reward: past and current status. *Trends in Neuroscience* 22, 521–527.
- Tongiorgi E, Armellin M, Giulianini PG, Bregola G, Zucchini S, Paradiso B, Steward O, Cattaneo A, Simonato M** (2004). Brain-derived neurotrophic factor mRNA and protein are targeted to discrete dendritic laminae by events that trigger epileptogenesis. *Journal of Neuroscience* 24, 6842–6852.
- Tongiorgi E, Righi M, Cattaneo A** (1997). Activity-dependent dendritic targeting of BDNF and TrkB mRNAs in hippocampal neurons. *Journal of Neuroscience* 17, 9492–9505.
- Tongiorgi E, Righi M, Cattaneo A** (1998). A non-radioactive in situ hybridization method that does not require RNase-free conditions. *Journal of Neuroscience Methods* 85, 129–139.
- Tropea D, Capsoni S, Tongiorgi E, Giannotta S, Cattaneo A, Domenici L** (2001). Mismatch between BDNF mRNA and protein expression in the developing rat visual cortex. *European Journal of Neuroscience* 13, 709–721.
- Vakili K, Pillay SS, Lafer B, Fava M, Renshaw PF, Bonello-Cintron CM, Yurgelun-Todd DA** (2000). Hippocampal volume in primary unipolar major depression: a magnetic resonance imaging study. *Biological Psychiatry* 47, 1087–1090.
- Van Hoomissen JD, Chambliss HO, Holmes PV, Dishman RK** (2003). Effects of chronic exercise and imipramine on mRNA for BDNF after olfactory bulbectomy in rat. *Brain Research* 974, 228–235.
- Zarate Jr. CA, Payne JL, Singh J, Quiroz JA, Luckenbaugh DA, Denicoff KD, Charney DS, Manji HK** (2004). Pramipexole for bipolar II depression: a placebo-controlled proof of concept study. *Biological Psychiatry* 56, 54–60.