Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pergamon
Neuropharmacology 45 (2003) 553–563
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Differential regulation of Brain Derived Neurotrophic Factor transcripts by antidepressant treatments in the adult rat brain

Brian G. Dias [a], Sunayana B. Banerjee [a], Ronald S. Duman [b], Vidita A. Vaidya [a,*]

[a] *Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Colaba, Mumbai, 400 005, India*
[b] *Department of Psychiatry, Yale University School of Medicine, CT, USA*

Received 2 January 2003; received in revised form 29 April 2003; accepted 8 May 2003

Abstract

Antidepressants are known to increase brain derived neurotrophic factor (BDNF) mRNA in the adult rat brain. The BDNF gene has four differentially regulated promoters that generate four transcript forms, each containing a unique non-coding 5′ exon (exon I–IV) and a common 3′ coding exon. Using in situ hybridization with exon-specific riboprobes, we have examined whether diverse classes of antidepressants recruit a single or multiple BDNF promoters to regulate BDNF mRNAs. The antidepressants tested were electroconvulsive seizure (ECS) and the pharmacological antidepressants tranylcypromine, desipramine and fluoxetine. The effects of both acute and chronic ECS were the most prominent on exon I and II containing BDNF mRNAs in hippocampal and cortical subfields. Chronic ECS enhanced the acute induction of exon I, II and IV mRNAs but did not influence the acute upregulation of exon III mRNAs. Acute pharmacological antidepressants resulted in region-specific decreases in distinct exon-specific BDNF transcripts. In contrast, chronic administration with tranylcypromine and desipramine enhanced exon II and exon III mRNAs, respectively, in discrete hippocampal and cortical subfields. Chronic fluoxetine treatment did not have a significant effect on the exon-specific BDNF transcripts. The results indicate that distinct antidepressants differentially regulate BDNF mRNAs through a region-specific recruitment of the four BDNF promoters and suggest that diverse signaling mechanisms may be recruited to regulate BDNF transcripts.

Keywords: Brain derived neurotrophic factor exon; Fluoxetine; Desipramine; Electroconvulsive seizure; Tranylcypromine; Neurotrophin

1. Introduction

Depression is a complex, heterogeneous disorder that affects approximately 15% of the population at some stage during their lifetime. The pathogenesis of this disease as well as the mechanisms of action underlying currently available antidepressant treatments are as yet unclear (Wong and Licinio, 2001). Although the acute sites of action for several classes of antidepressant treatments differ, these treatments all require chronic administration to exert their therapeutic benefits. This has led to the suggestion that adaptations in response to chronic treatment may underlie the benefits of antidepressants. One such common adaptation may involve altered structural plasticity within the nervous system, such as enhanced hippocampal neurogenesis and mossy fiber sprouting (Vaidya et al., 1999; Malberg et al., 2000). Amongst the common targets of several classes of antidepressants is the neurotrophin, brain derived neurotrophic factor (BDNF) that has been implicated in antidepressant-induced structural plasticity (Vaidya and Duman, 2001).

BDNF plays a critical role during development and retains a profound influence on structural and synaptic plasticity in the mature brain (Thoenen, 1995; Kafitz et al., 1999). A role for BDNF in the actions of antidepressants is supported by several lines of evidence (Altar, 1999). First, chronic antidepressant treatments enhance BDNF mRNA and protein expression within distinct regions of the adult rat brain (Nibuya et al., 1995; Lindefors et al., 1995; Angelucci et al., 2002) and enhanced BDNF immunoreactivity has also been observed in postmortem tissue from antidepressant treated patients (Chen

* Corresponding author. Tel.: +91-22-2280-4545; fax: +91-22-2280-4610/4611.
E-mail address: vvaidya@tifr.res.in (V.A. Vaidya).

0028-3908/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0028-3908(03)00198-9

et al., 2001). Second, antidepressant pre-treatment prevents stress-induced decreases in hippocampal BDNF expression (Nibuya et al., 1995). Third, BDNF infusion exerts antidepressant-like effects in two distinct animal models of depression (Siuciak et al., 1997; Shirayama et al., 2002). In addition, BDNF has been reported to enhance ongoing adult neurogenesis (Pencea et al., 2001) and contribute to hippocampal mossy fiber sprouting (Vaidya et al., 1999). Taken together, these studies provide evidence to support the hypothesis that BDNF may play a critical role in the actions of antidepressant treatments.

Given the important role of BDNF in neuronal plasticity and the actions of antidepressant treatments, the mechanisms and the manner in which BDNF expression is regulated are of considerable interest. The rat BDNF gene can generate eight distinct BDNF transcripts through the alternate splicing of four unique 5′ exons (exon I–IV) to a common 3′ exon (exon V) (Timmusk et al., 1993) and the use of two possible polyadenylation sites. The 5′ exons, each with their separate promoters, remain untranslated and it is only the common 3′ exon that generates mature BDNF protein. These multiple BDNF promoters are recruited to generate a region-specific basal expression of different BDNF transcripts and are also differentially sensitive to stimuli (Bishop et al., 1994; Kokaia et al., 1994). Specific exon-containing BDNF mRNAs are differentially regulated by both physiological stimuli such as diurnal rhythm and exercise, and pathophysiological insults such as seizures, ischemia and hypoglycemic coma (Kokaia et al., 1994; Oliff et al., 1998; Berchtold et al., 1999).

It is unknown at present if different antidepressant treatments regulate BDNF gene expression through the recruitment of the same BDNF promoter or whether multiple BDNF promoters are differentially targeted by specific antidepressants to enhance BDNF gene expression. An emerging hypothesis suggests that a common target of antidepressants may be an increase in cAMP response element (CRE)-mediated transcription through enhanced levels of the transcription factor cAMP response element binding protein (CREB) (Nibuya et al., 1996; Thome et al., 2000). Increases in CREB may then underlie the upregulation of BDNF expression by binding to CRE-elements and enhancing transcription (Conti et al., 2002). There is evidence for a CRE element within the exon III BDNF promoter and CREB has been shown to regulate calcium responsiveness of exon III containing BDNF mRNAs (Tao et al., 1998). Given the complexity of the BDNF gene, it is important to examine the manner in which distinct BDNF transcripts are regulated by antidepressant treatments. At present, only a single study has examined the regulation of exon I and II-containing BDNF transcripts following chronic treatment with the antidepressant, tranylcypromine (Russo-Neustadt et al., 2000).

In the present study, the influence of diverse classes of antidepressant treatments including electroconvulsive seizure (ECS), the norepinephrine selective reuptake inhibitor desipramine, the monoamine oxidase inhibitor tranylcypromine and the serotonin selective reuptake inhibitor fluoxetine on different BDNF transcripts was assessed using radioactive in situ hybridization with riboprobes specific to each of the different 5′ exons (exon I–IV). The results indicate that diverse classes of antidepressants differentially recruit multiple promoters to drive the expression of distinct BDNF mRNAs in a region-specific manner.

2. Materials and methods

2.1. Animal treatment paradigms

Male Sprague-Dawley rats (200–250 g) bred in our animal-breeding colony were used in all experiments. Animals were group housed and maintained on a 12 h light–dark cycle with access to food and water ad libitum. All animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the TIFR Institutional Animal Ethics Committee. Animals received ECS via earclip electrodes (Stoelting, USA) (70 mA, 0.5 s) or sham treatment (application of earclip electrodes without electrical stimulation). For the acute ECS study, animals ($n = 5$/group) received a single sham or ECS treatment and were sacrificed 2 h later. For the chronic ECS paradigm, animals ($n = 5$/group) received sham or ECS treatment once daily for 10 consecutive days and were sacrificed 2 h after the last ECS treatment. In the ECS time-course experiment, rats received a single sham or ECS treatment and were sacrificed 2, 4, 6, 8 and 24 h following ECS administration. Each individual time-point had a separate sham group that was sacrificed along with the ECS group ($n = 3$/group).

For the chronic pharmacological antidepressant treatments, groups of animals received desipramine (15 mg/kg) ($n = 4$/group), tranylcypromine (7.5 mg/kg for 7 days and then 10 mg/kg for 14 days) ($n = 3$/group), fluoxetine (5 mg/kg) ($n = 5$/group) or vehicle (0.9% saline) treatment once daily for 21 days through intraperitoneal (i.p.) injection. These were separate experiments, each with their distinct vehicle treatment groups. Animals in the chronic antidepressant drug study were sacrificed 2 h after the last drug treatment. In the acute antidepressant study, animals received a single vehicle, desipramine (15 mg/kg) or tranylcypromine (7.5 mg/kg) ($n = 4$/group) treatment and were sacrificed 2 h later. In a separate experiment, animals received a single injection of vehicle or fluoxetine (5 mg/kg) ($n = 4$/group) and were sacrificed 2 h later. The choice of dose and

treatment regimen for the pharmacological antidepressants was based on previous studies (Nibuya et al., 1995, 1996; Takahashi et al., 1999) that indicated a significant regulation of BDNF exon V mRNA, CREB mRNA and CRE binding following chronic administration (21 days). After decapitation, brains were rapidly removed and frozen on dry ice and stored at −70 °C prior to cryostat sectioning and in situ hybridization analysis. The pharmacological antidepressants desipramine, fluoxetine and tranylcypromine used in this study were obtained from Sigma-RBI (St. Louis, USA).

2.2. In situ hybridization

In situ hybridization was carried out as previously described (Gall et al., 1995; Lauterborn et al., 1996). In brief, 14 μm thick coronal sections were cut on the cryostat and thaw mounted onto ribonuclease free Probe-on plus slides (Electron Microscopy Services, USA). Slides were then fixed in 4% paraformaldehyde, acetylated and dehydrated prior to storage at −70 °C. Rat exon-specific BDNF cRNA probes were generated from transcription competent plasmids provided by Dr. Lauterborn (University of California, Irvine). Antisense riboprobes specific to exon I (378 bp; bases 787–1165), exon II (468 bp; bases 1761–2229), exon III (391 bp; bases 636–1027) and exon IV (350 bp; bases 1730–2080) were transcribed from *Pst*I digested pVEI, *Pvu*II digested pVEII, *Hind*III digested pVEIII using T7 RNA polymerase and from *Pvu*II digested pVEIV using the T3 RNA polymerase as described previously (Lauterborn et al., 1996). All cRNA probes were transcribed using ^{35}S-labeled UTP (Amersham, Buckinghamshire, UK). Slides were incubated for 20–24 h at 60 °C with hybridization buffer (50% formamide, 1X SSC, 25X Denhardts solution, 40 mM dithiothreitol, 150 μg/ml yeast tRNA, 10% dextran sulphate, 400 μg/ml salmon sperm DNA) and ^{35}S-UTP labeled exon-specific riboprobes at a concentration of 1×10^6 cpm/250 ul. After hybridization, the tissue was washed in 4X SSC at 60 °C, treated with RNase A (20 μg/ml) at 45 °C for 30 min, followed by stringent washes in decreasing concentrations of SSC with a final wash in 0.5X SSC at 60 °C. Slides were air dried and exposed to Hyperfilm β-max (Amersham, UK) for 6 weeks. Sense riboprobes for the different BDNF exons, or a ribonuclease (40 μg/ml at 37 °C for 30 min) pre-hybridization wash, did not yield significant hybridization (data not shown) confirming the specificity of the signal observed with the exon-specific antisense riboprobes.

2.3. Quantitation and data analysis

Levels of BDNF transcripts containing specific exons (I–IV) were analyzed using the Macintosh-based Scion Image software (Scion, USA). To correct for non-linearity, ^{14}C standards were used for calibration. The dentate gyrus granule cell layer, CA3, CA1, CA4 pyramidal cell layers, piriform cortex, motor cortex, amygdala and the outer and inner layers of neocortex were analyzed. An equivalent area was outlined for each sample and optical density measurements from both sides of 3–4 individual sections from each animal were analyzed, from which the mean value was calculated. Results were subjected to statistical analysis using Student's *t*-test for experiments with two groups, or Analysis of Variance (ANOVA) followed by a Tukey-Kramer post-hoc test for experiments with three groups. Differences were considered to be statistically significant at p values < 0.05.

3. Results

The effect of acute and chronic antidepressant treatments on the expression of different BDNF transcripts was examined using in situ hybridization with sequence-specific riboprobes for each BDNF exon (exon I–IV). The distribution and relative levels of the exon-specific BDNF transcripts under basal conditions was similar to that reported previously (Timmusk et al., 1993; Lauterborn et al., 1996). Densitometric analysis to quantify levels of different BDNF mRNAs was carried out in the dentate gyrus (DG), CA3, CA1, CA4, piriform cortex, motor cortex, amygdala and the outer and inner layers of neocortex. Although all these regions were quantitated for each experiment, figures containing graphical representation of the levels of exon-specific BDNF mRNAs include primarily the hippocampal subfields (DG, CA1, CA3 and CA4) and those cortical regions where an effect was observed following antidepressant treatments.

3.1. Influence of acute electroconvulsive seizure on different exon-containing BDNF mRNAs in the rat brain

The influence of acute ECS on the expression of distinct exon-containing transcripts is shown in the representative autoradiograms in Fig. 1, and the quantitation of the changes observed following acute ECS are presented in Fig. 2. Acute ECS treatment led to a large, pronounced induction in exon I mRNA in comparison to the induction of exon II, III and IV containing BDNF transcripts observed following acute ECS (Fig. 2). The most prominent increase in exon I mRNA was a 3-fold induction in the DG region. Exon I mRNA levels were also upregulated in the motor cortex, piriform cortex and amygdala. The induction of exon I mRNA in motor cortex appeared restricted to the superficial layers (Fig. 1A). Significant increases were also observed in exon II mRNA levels within the DG, piriform cortex and amygdala (Fig. 2). Exon III containing transcripts showed a more ubiquitous basal expression and doubled in the DG

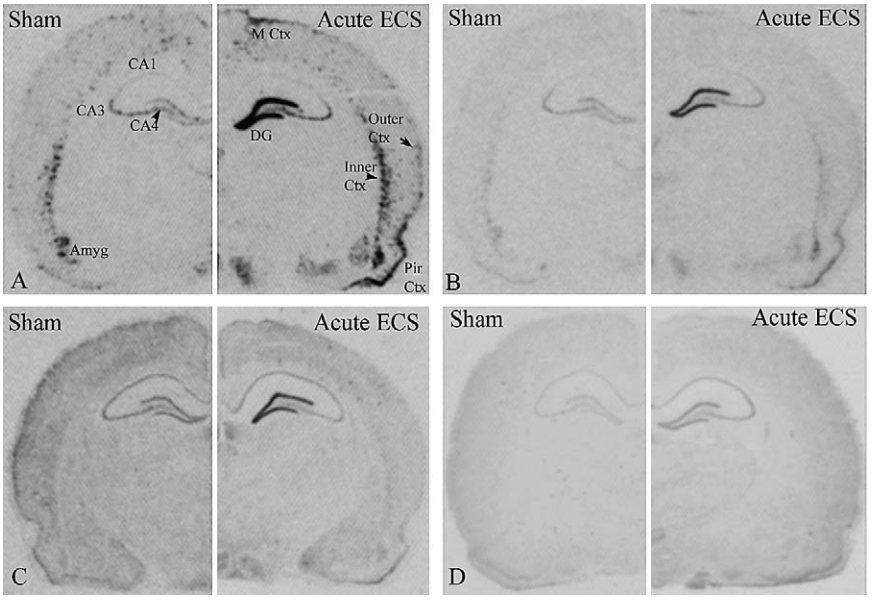

Fig. 1. Regulation of BDNF transcripts containing specific exons (I–IV) in hippocampal and cortical subfields following acute ECS. Rats were administered sham or acute ECS treatment and levels of BDNF transcripts were determined by in situ hybridization using specific riboprobes for the different exons (I–IV). Representative autoradiograms from a sham and acute ECS treated animal for each of the four exon-containing BDNF transcripts are shown. (A) exon I; (B) exon II; (C) exon III; (D) exon IV. The CA1, CA3, CA4 pyramidal cell layer, dentate gyrus granule cell layer (DG), amygdala (Amyg), motor Cortex (M Ctx), piriform ctx (Pir Ctx), inner and outer layers of neocortex are indicated in (A).

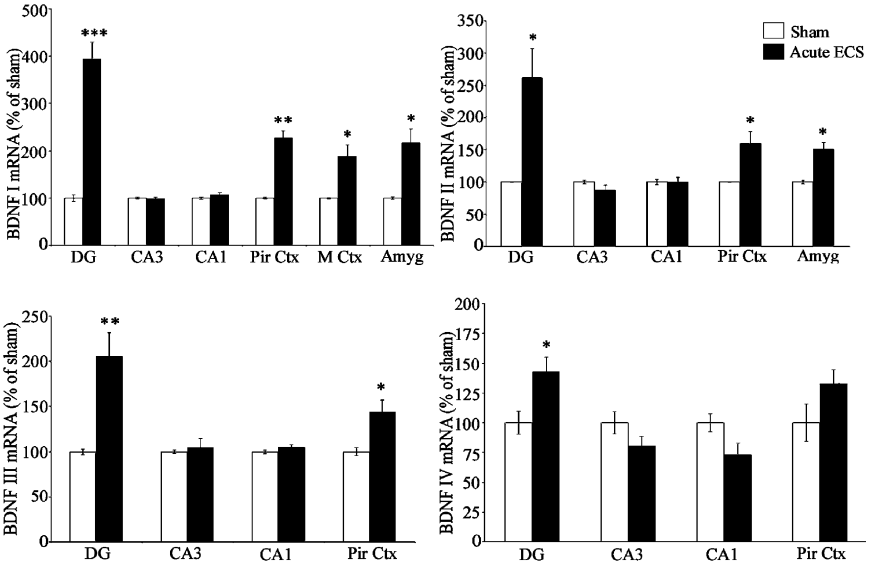

Fig. 2. Influence of acute ECS on the levels of specific exon-containing BDNF mRNAs in hippocampal and cortical subfields. Animals received a single sham or ECS treatment and were sacrificed 2 h later. Sections were subjected to in situ hybridization analysis using riboprobes specific to the distinct BDNF exons (I–IV). Quantitation of the levels of different BDNF transcripts (exon I–IV) were performed using densitometric analysis. Levels of BDNF exon-specific mRNA levels in the dentate gyrus (DG), CA3, CA1, piriform cortex (Pir Ctx), motor cortex (M Ctx) and amygdala (Amyg) are shown. The results are represented as percent of sham and are the mean ± SEM ($n = 5$/group). $*\ p < 0.05$, $**\ p < 0.01$, $***\ p < 0.001$ in comparison to sham treated controls (Student's *t*-test). The scales for the *y*-axis differ between graphs for distinct BDNF exon-containing mRNAs.

region following acute ECS administration. Although no changes were observed in the amygdala in exon III mRNAs (data not shown) a small but significant induction was observed in the piriform cortex. A significant increase in exon IV transcript levels was restricted to the DG region and no change in exon IV levels was observed in any other region following acute ECS. Although the expression of all BDNF transcripts (exon I–IV) was enhanced within the DG subfield of the hippocampus, the hippocampal CA pyramidal cell fields did not show any change in BDNF transcripts following acute ECS administration (Fig. 2).

3.2. Time-dependent regulation of exon-specific BDNF transcripts

The temporal regulation of the different exon-containing BDNF transcripts was examined at 2, 4, 6, 8 and 24 h following an acute ECS administration and each time-point had a distinct sham treatment group. The graph depicting distinct BDNF transcript levels at different time-points following a single ECS treatment (Fig. 3) is primarily restricted to those regions in which a significant effect was observed. The increase in exon I, II, III and IV mRNAs was maximal at 2 h and levels of all BDNF transcripts had returned to baseline by 24 h (Fig. 3). The ECS-induced upregulation in exon I mRNA seen in the DG and piriform cortex returned to baseline levels gradually, with significant increases over sham treated controls still evident at 6 h following treatment. In contrast, the upregulation of BDNF exon I mRNA in the amygdala and in the superficial layers of motor cortex showed a return to baseline by 4 h after treatment. Although the induction in all the exon-specific BDNF transcripts was maximal at 2 h, an exception was observed in the inner layers of neocortex which did not show any increase in exon I mRNA at 2 h and was observed to have a 50% increase over sham 8 h after seizure (Sham = 100 ± 7.28; Acute ECS + 8 h = 149.50 ± 11.94, $p < 0.05$, Student's *t*-test). This induction in exon I within the inner layers of neocortex returned to baseline by 24 h (data not shown). Exon II mRNA levels significantly enhanced at 2 h after ECS treatment also showed a gradual decline to baseline in the DG and piriform cortex with significantly increased levels still evident at 6 h post seizure treatment. However, the induction of exon II mRNA in the amygdala at 2 h after ECS returned to baseline at 4 h and a small but significant downregulation was observed 8 h after the ECS treatment. In contrast to the gradual decrease to baseline observed with both exon I and II containing transcripts, a rapid return to sham levels was observed in the exon III and IV mRNA levels following a significant induction at the 2 h time-point. In the piriform cortex exon III mRNA levels showed a bidirectional response with an increase observed 2 h post acute ECS and a decrease evident 6 h post seizure administration.

3.3. Regulation of exon-specific BDNF mRNA transcripts in the rat brain by chronic electroconvulsive seizures

The influence of chronic ECS (once daily for 10 consecutive days) on the distinct exon containing BDNF

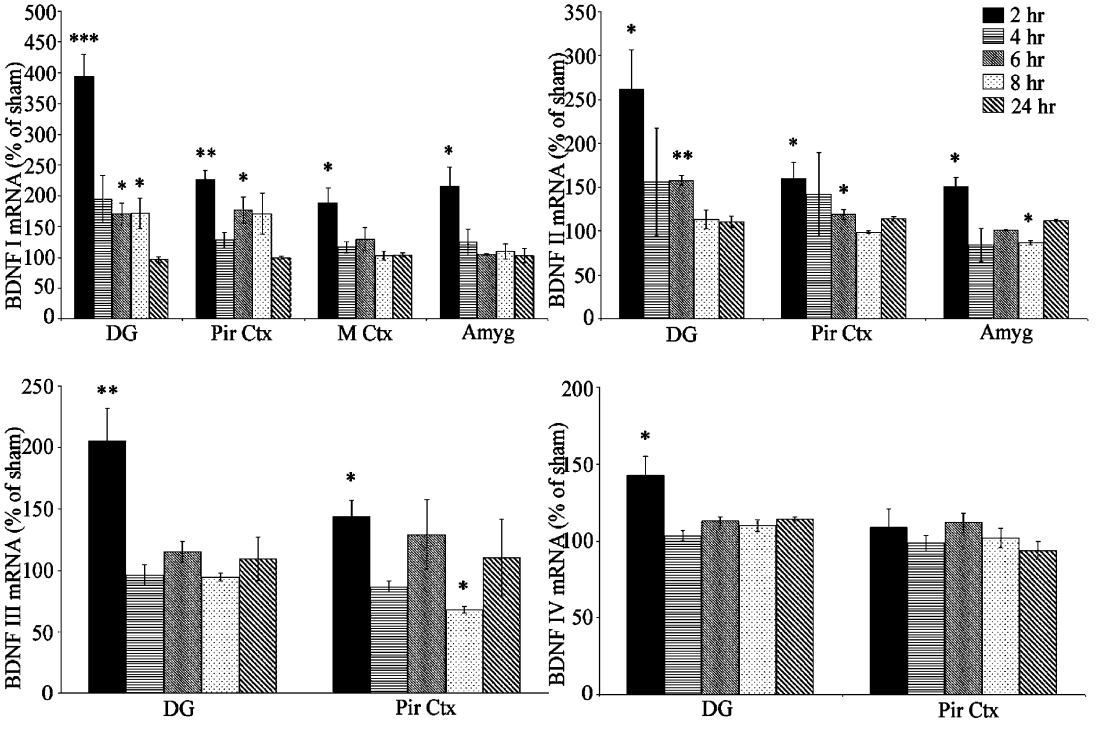

Fig. 3. Time-dependent regulation of different exon-containing BDNF transcripts following acute ECS administration. Animals received a single sham or ECS treatment and were sacrificed 2, 4, 6, 8 and 24 h after treatment. Each time point had its own separate sham group ($n = 3$/group). Levels of BDNF transcripts containing different exons (I–IV) were determined using in situ hybridization and were quantitated using densitometric analysis. The levels of different BDNF mRNAs in the dentate gyrus (DG), piriform cortex (Pir Ctx), motor cortex (M Ctx) and amygdala (Amyg) are represented. The upregulation of different BDNF transcripts follows a distinct temporal pattern following ECS treatment. The results are represented as percent of sham and are the mean ± SEM ($n = 3$/group). $* p < 0.05$, $** p < 0.01$, $*** p < 0.001$ in comparison to sham (Student's *t*-test). The scales for the *y*-axis differ between graphs.

mRNA transcripts was examined (Fig. 4). Regions that did not respond to a single administration of ECS showed significant inductions in specific BDNF mRNAs following chronic ECS administration. Chronic ECS treatment significantly induced exon I mRNA levels in the DG, CA4, piriform cortex, motor cortex and outer layers of neocortex. The induction of BDNF I mRNA in the DG, piriform and motor cortex was potentiated in comparison to the induction observed following a single ECS treatment (see Figs. 2 and 4). Although exon I mRNA levels showed a trend towards an increase in the amygdala following chronic ECS, this was not significant ($p = 0.09$). Chronic ECS treatment induced increases in exon II transcripts within similar regions as the induction of exon I mRNAs, and in addition were upregulated within the amygdala and CA3 hippocampal subfield. The induction of exon II mRNA was potentiated after chronic ECS treatment in all regions examined in comparison to the upregulation of exon II mRNAs following acute ECS (see Figs. 2 and 4). In contrast, exon III containing BDNF transcripts were unique in their response to chronic ECS treatment. Following chronic ECS, the upregulation of exon III mRNAs was restricted to the DG and no changes were observed in any other regions including the piriform cortex, which responded to acute ECS with a significant induction (see Figs. 2 and 4). Unlike the effects observed with exon I and II mRNAs where chronic ECS treatment led to an even greater increase in levels of transcripts, no such trend was observed in exon III containing transcripts (see Figs. 2 and 4). Chronic ECS mediated induction of exon IV mRNAs was restricted to the DG and chronic ECS resulted in an increase in the acute induction of exon IV containing BDNF mRNA in the DG.

3.4. Effects of acute and chronic desipramine, tranylcypromine and fluoxetine administration on exon-specific BDNF mRNA transcripts in the rat brain

The influence of single or repeated (once daily for 21 days) administration of the pharmacological antidepressants desipramine, tranylcypromine and fluoxetine on specific exon-containing BDNF transcripts was examined using in situ hybridization (Fig. 5). Acute tranylcypromine and desipramine treatment both led to a significant reduction in exon II containing BDNF transcripts within the DG region (Fig. 5). Acute desipramine and tranylcypromine treatments did not influence exon I, III and IV mRNAs in any of the other brain regions examined. In contrast, acute treatment with fluoxetine resulted in a small but significant decrease of exon III mRNA in the amygdala and exon IV mRNA in the CA1 pyramidal cell layer.

Chronic treatment with tranylcypromine led to a significant induction in exon II mRNA levels within the hippocampal CA subfields and in the piriform cortex (Fig. 6). Although there was no significant regulation of BDNF exon I mRNA following chronic tranylcypromine treatment, it is possible that additional experiments may reveal a small effect of tranylcypromine on the BDNF exon I transcript. Chronic desipramine administration resulted in small but significant increases of exon III

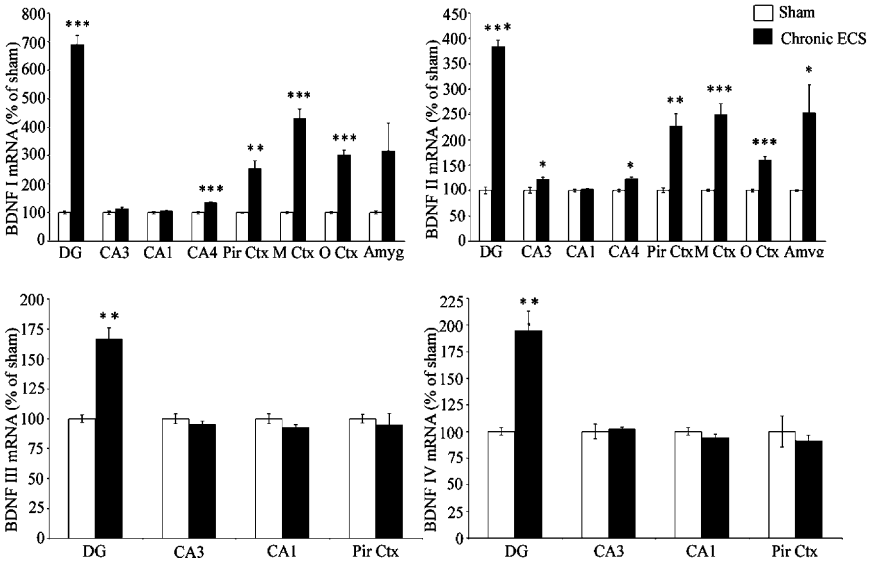

Fig. 4. Influence of chronic ECS administration on levels of different exon-containing (I–IV) BDNF transcripts in hippocampal and cortical subfields. Animals received a sham or ECS treatment once daily for 10 consecutive days and were sacrificed 2 h after the last treatment. Levels of distinct exon-specific BDNF mRNAs were quantitated using densitometric analysis. The levels of different BDNF mRNAs (exon I–IV) in the dentate gyrus (DG), CA3, CA1, CA4, piriform cortex (Pir Ctx), motor cortex (M Ctx), outer cortex (O Ctx) and amygdala (Amyg) are represented. The results are represented as percent of sham and are the mean ± SEM ($n = 5$/group). $*\ p < 0.05$, $**\ p < 0.01$, $***\ p < 0.001$ in comparison to sham (Student's *t*-test). The scales for the *y*-axis differ between graphs.

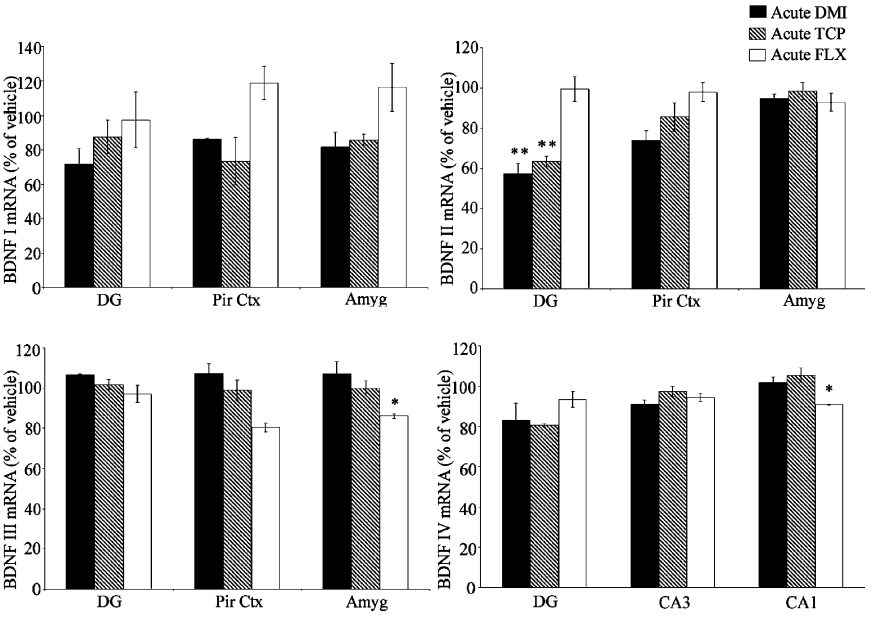

Fig. 5. Regulation of different exon-containing BDNF transcripts following acute antidepressant drug treatment. Animals were administered vehicle, tranylcypromine (TCP) or desipramine (DMI) ($n = 4$/group), and were sacrificed 2 h after the injection. In a separate experiment, animals received vehicle or fluoxetine (FLX) administration ($n = 4$/group), and were sacrificed 2 h post treatment. In situ hybridization for BDNF transcripts using exon-specific riboprobes was carried out and transcript levels were quantified using densitometric analysis. Exon I, II and III mRNA levels within the dentate gyrus (DG), amygdala (Amyg) and piriform cortex (Pir Ctx) and exon IV mRNA levels in the DG, CA1 and CA3 subfields are represented. Results are expressed as percent of vehicle and represented as mean ± SEM ($n = 4$/group). $*\ p < 0.05$, $**\ p < 0.01$, $***\ p < 0.001$ in comparison to vehicle. Experiment with TCP and DMI: ANOVA; Tukey-Kramer post-hoc test; experiment with FLX: Student's *t*-test. The scales for the *y*-axis differ between graphs for distinct BDNF exon-containing mRNAs.

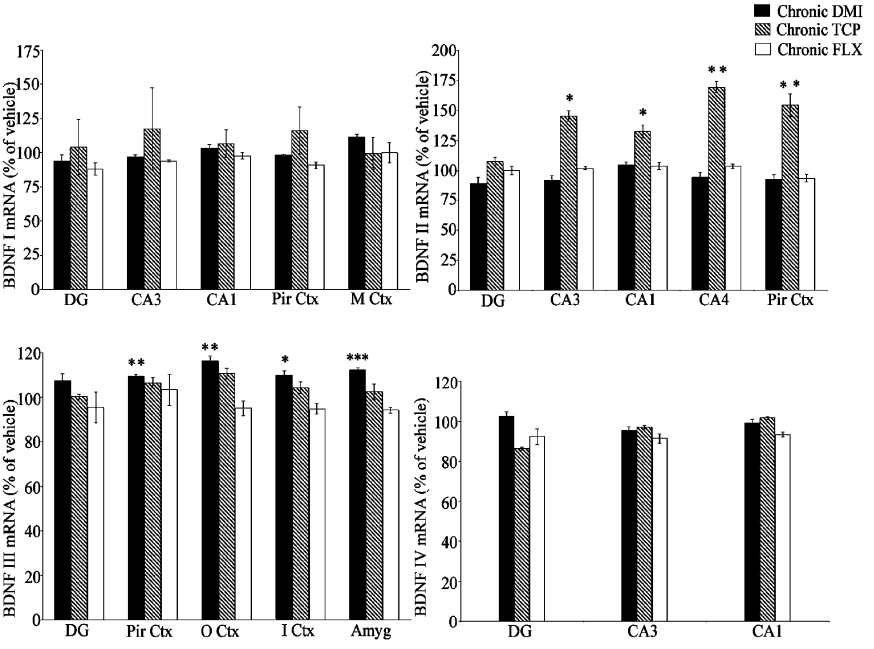

Fig. 6. Influence of chronic antidepressant drug treatments on specific exon-containing BDNF mRNA transcripts. In three distinct experiments, animals were administered either vehicle and tranylcypromine (TCP) ($n = 3$/group), desipramine (DMI) ($n = 4$/group) or fluoxetine (FLX) ($n = 5$/group) once daily for 21 days and sacrificed 2 h after the last injection. These were separate experiments each with their own vehicle treatment group. In situ hybridization was performed using exon-specific riboprobes and levels of BDNF transcripts were determined using densitometry. Levels of exon-specific BDNF mRNAs in the dentate gyrus (DG), CA1, CA3, CA4 pyramidal fields, piriform cortex (Pir Ctx), motor cortex (M Ctx), amygdala (Amyg) and outer and inner layers of neocortex (O Ctx and I Ctx) are represented. Results are expressed as percent of vehicle and represented as the mean ± SEM. $*\ p < 0.05$, $**\ p < 0.01$, $***\ p < 0.001$ in comparison to vehicle (Student's *t*-test). The scales for the *y*-axis differ between graphs for distinct BDNF exon-containing mRNAs.

transcripts in the piriform cortex, outer and inner layers of neocortex and amygdala (Fig. 6). In contrast, chronic fluoxetine treatment did not significantly influence the expression of any of the specific exon-containing BDNF transcripts within the regions examined.

4. Discussion

The results of this study demonstrate that diverse classes of antidepressants exhibit a region-specific, differential regulation of exon-containing BDNF mRNAs. Each 5′ exon is linked to a unique promoter and our results suggest that antidepressants differentially recruit exon-specific promoters to regulate BDNF mRNA expression. These results suggest a more complex regulation of BDNF expression by antidepressants than revealed by previous studies that did not distinguish among multiple BDNF transcripts.

Previous studies (Nibuya et al., 1995; Lindefors et al., 1995) using probes to the coding exon (V) that does not distinguish among BDNF transcripts, indicate a marked ECS-induced upregulation of BDNF expression in hippocampal and cortical regions. The present study indicates that while all four exon-containing transcripts differentially contribute to the upregulation of BDNF mRNA in the dentate gyrus, the enhanced BDNF expression in the amygdala, piriform cortex and neocortex primarily results from an increased expression of exon I and II containing BDNF transcripts. Although ECS resulted in a more prominent induction of exon I than exon II mRNAs, the regional and temporal pattern of regulation for both exons were remarkably similar. Previous reports (Lauterborn et al., 1996; Lauterborn et al., 1998) have suggested that the mechanisms regulating the expression of exon I and exon II-containing transcripts are similar, and distinct from those underlying the regulation of exon III and IV mRNAs. The promoters of exon I and II are located in close proximity suggesting the possibility of shared regulatory regions (Timmusk et al., 1993). Our results suggest that exon I and II transcripts may be regulated in a pairwise manner by ECS treatments in specific regions. The mechanisms underlying such a coordinated regulation of exon-specific BDNF mRNAs by ECS administration if any, are at present unknown.

Several seizure paradigms have been reported to predominantly induce exon I and III-containing BDNF mRNAs. Although the primary BDNF promoters that are targeted by kindling, kainate, bicuculline and pilocarpine induced seizures are those upstream of BDNF exon I and III, the regional pattern of the exon-specific BDNF transcripts induced is quite distinct (Metsis et al., 1993; Kokaia et al., 1994). This suggests that despite the generalized increase in neuronal activity that occurs in all seizure paradigms, specific differences in patterns of activation of glutamatergic and non-glutamatergic pathways, as well as mode of calcium influx might underlie the regional differences seen in the regulation of BDNF transcripts. The major striking difference between the regulation of BDNF transcripts by ECS and other seizure paradigms is that ECS predominantly recruits exon I and II transcripts, in contrast to the exon I and III transcripts that are the prominent targets of other seizure paradigms. This difference is intriguing given previous reports that BDNF exon III transcripts are particularly sensitive to neuronal trauma such as that induced by hypoglycemic coma, ischemia as well as epileptic seizure models (Timmusk et al., 1993; Kokaia et al., 1994). Our study indicates that the ECS paradigm, which acts as a powerful antidepressant and is distinct from paradigms that model epilepsy or cause neuronal damage, also differs in the regulation of exon-specific BDNF transcripts by recruiting mainly exon I and II promoters. It is possible that the dramatic inductions seen in exon III transcripts following epileptic seizure models and other neuronal insults involves pathways recruited by excitotoxic damage, and given that ECS does not result in excitotoxic cell death (Vaidya et al., 1999) such a prominent regulation of BDNF exon III transcripts is absent following acute and chronic ECS.

The present study also examined the effects of acute and chronic pharmacological antidepressant treatments on exon-specific BDNF transcripts. Our results revealed that acute tranylcypromine and desipramine administration led to marked decreases of exon II transcripts in the dentate gyrus. Acute fluoxetine treatment resulted in small decreases in exon III and IV mRNAs in the amygdala and CA1, respectively. Previous studies have suggested that an acute induction in serotonin and norepinephrine levels results in a down-regulation of hippocampal BDNF V mRNA (Hutter et al., 1996; Zetterstrom et al., 1999). Such an acute induction in serotonin and norepinephrine levels may also underlie the down-regulation of specific exon-containing BDNF transcripts seen in our study following acute antidepressant treatment.

In contrast following chronic administration, tranylcypromine upregulated exon II mRNAs in all hippocampal CA subfields and the piriform cortex. Chronic desipramine treatment caused small but significant increases of exon III transcripts in the amygdala, piriform cortex and neocortex. However, chronic fluoxetine treatment did not significantly influence any of the exon-specific BDNF transcripts. Previous studies using probes that did not distinguish among multiple BDNF transcripts have indicated that chronic tranylcypromine increased, whereas acute tranylcypromine treatment decreased (Zetterstrom et al., 1999) or did not influence (Nibuya et al., 1995), hippocampal BDNF mRNA. A recent report (Russo-Neustadt et al., 2000) examining the influence of chronic tranylcypromine on exon I and II mRNAs

revealed increases in hippocampal exon I, but not exon II, transcripts. In contrast, our results suggest that the regulation of hippocampal BDNF mRNA by acute and chronic tranylcypromine treatment is mediated through the recruitment of the exon II, but not the exon I, promoter. The differences observed between our results and previous reports (Russo-Neustadt et al., 2000) are likely to arise from the different time points for analysis, 2 or 21 h following the last tranylcypromine treatment, respectively. Taken together, these studies suggest that chronic tranylcypromine treatment exhibits a differential temporal and spatial regulation of both exon I and II BDNF mRNAs. Previous reports (Nibuya et al., 1995; Conti et al., 2002) using riboprobes to exon V have shown that chronic desipramine treatment results in small but significant increases in cortical and hippocampal BDNF mRNA expression. Our results indicate that chronic desipramine recruits the exon III promoter to enhance cortical BDNF expression but does not regulate hippocampal exon-specific BDNF transcripts. It is possible that the small change seen in total hippocampal BDNF mRNA following chronic desipramine in previous studies may not be evident in our study using exon-specific BDNF riboprobes.

Contrary to our expectations, we did not observe an increase in any of the exon-specific BDNF transcripts following chronic fluoxetine treatment. The discrepancy between our findings using BDNF exon-specific riboprobes and previous results (Nibuya et al., 1995, 1996) may arise due to the differences in methodology and treatment paradigm. First, the study by Nibuya et al. (1995) used the serotonin selective reuptake inhibitor (SSRI) sertraline and with a treatment paradigm similar to that used in our study reported a small 20% induction in hippocampal BDNF V mRNA. It is possible that such a small induction in BDNF V mRNA may not be picked up in an approach that distinguishes among multiple BDNF transcripts. Second, the study by Nibuya et al. (1996) used fluoxetine at the same dose utilized in our study but examined BDNF V mRNA 18 h following chronic treatment, a time-point at which they report an upregulation. The discrepancies in our study and that of Nibuya et al. (1996) may arise due to differences in the time-point used. However, it is important to point out that our studies are in agreement with recent reports using both low and high doses of chronic fluoxetine treatment that have failed to observe an upregulation in BDNF V mRNA (Miro et al., 2002; Conti et al., 2002). Although the discrepancies observed in these studies when using fluoxetine may arise simply due to different doses, time points and methodology used, one cannot rule out the possibility that SSRIs may not be as robust as other classes of pharmacological antidepressants in their ability to upregulate BDNF transcripts following chronic administration.

The signaling pathways and transcriptional mechanisms underlying the regulation of exon-specific BDNF expression by diverse classes of antidepressants are at present unknown. Our results indicate that chronic ECS predominantly recruits exon I and II promoters, whilst chronic tranylcypromine and desipramine treatments recruit exon II and III promoters, respectively. Previous studies have suggested that the cyclic AMP (cAMP) signal transduction cascade is a common post-receptor target for chronic antidepressant treatments, which may then influence the expression of target genes like BDNF through the transcription factor CREB (Nibuya et al., 1996; Thome et al., 2000; Conti et al., 2002). There is a CREB binding site upstream of the exon III promoter (Tao et al., 1998) and it is intriguing to note that a previous study (Conti et al., 2002) reported that CREB deficient mice failed to show increases in BDNF expression following chronic desipramine. Our results indicate that chronic desipramine recruits the exon III promoter providing further evidence that CREB may play an important role in the regulation of BDNF mRNA by desipramine. However, chronic ECS showed a relatively small increase of exon III transcripts restricted to the hippocampus, and chronic tranylcypromine did not influence exon III mRNAs. This suggests that in addition to the cAMP–CREB cascade, diverse classes of antidepressants may differentially recruit signaling pathways and transcription factors in a region-specific manner to regulate multiple BDNF transcripts. Indeed, chronic antidepressant treatments have been reported to regulate the transcription factors c-fos and zif-268 (Bjartmar et al., 2000; Morinobu et al., 1997) for which putative binding sites have been reported in the BDNF gene (Nakayama et al., 1994) suggesting that they may contribute to the regulation of BDNF expression.

The physiological consequences of differentially regulated, exon-specific BDNF transcripts are as yet unknown. Although all BDNF transcripts contain the same coding sequence, distinct 5′ untranslated exons through effects on RNA-protein interactions could influence stability, translatability and trafficking of BDNF mRNAs. The antidepressant-mediated increases in exon-specific BDNF mRNAs are most likely to reflect a differential recruitment of cognate promoters, but the contribution of altered transcript stability cannot be ruled out and requires further examination. In addition, 5′ exons through secondary structure formation may influence translatability indicating that multiple BDNF transcripts provide for the control of BDNF expression at both the transcription and translation level (Timmusk et al., 1993). The possibility of differential targeting of BDNF transcripts within a cell is particularly intriguing. BDNF has been reported to act as both a retrograde and anterograde factor, released by postsynaptic and presynaptic neurons, respectively (Heersen and Segal, 2002). It is possible that BDNF transcripts may be spatially targeted and studies have shown dendritic localization of

BDNF mRNA in hippocampal neurons (Crino and Eberwine, 1996; Righi et al., 2000). There is evidence that spatially distinct signaling pathways may result in location-specific biological responses to BDNF (Lom and Cohen-Cory, 1999). Although highly speculative, one can envisage a complex regulatory situation where differential transcription, trafficking and translation of multiple BDNF transcripts may contribute to the location-specific structural plasticity seen following antidepressant treatments, such as granule cell axonal sprouting (Vaidya et al., 1999), dendritic remodeling (Norrholm and Ouimet, 2001) and subgranular zone neurogenesis (Malberg et al., 2000). Future studies are required to unravel the mechanisms that underlie antidepressant-mediated regulation of exon-specific BDNF transcripts, as well as the physiological significance of these multiple transcripts and their contribution to the effects of antidepressant treatments.

Acknowledgements

This work was supported by a SERC grant from the Department of Science and Technology, Government of India.

References

Altar, C.A., 1999. Neurotrophins and depression. Trends in Pharmacological Sciences 20, 59–61.

Angelucci, F., Aloe, L., Jimenez-Vasquez, P., Mathe, A.A., 2002. Electroconvulsive stimuli alter the regional concentrations of nerve growth factor, brain-derived neurotrophic factor, and glial cell line-derived neurotrophic factor in adult rat brain. Journal of ECT 18 (3), 138–143.

Berchtold, N.C., Oliff, H.S., Isackson, P., Cotman, C.W., 1999. Hippocampal BDNF mRNA shows a diurnal regulation, primarily in the exon III transcript. Molecular Brain Research 71, 11–22.

Bishop, J.F., Mueller, G.P., Mouradian, M.M., 1994. Alternate 5′ exons in the rat brain-derived neurotrophic factor gene: differential patterns of expression across brain regions. Molecular Brain Research 26, 225–232.

Bjartmar, L., Johansson, I.M., Marcusson, J., Ross, S.B., Seckl, J.R., Olsson, T., 2000. Selective effects on NGFI-A, MR, GR and NGFI-B hippocampal mRNA expression after chronic treatment with different subclasses of antidepressants in the rat. Psychopharmacology 151, 7–12.

Chen, B., Dowlatshahi, D., MacQueen, G.M., Wang, J.F., Young, L.T., 2001. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biological Psychiatry 50, 260–265.

Conti, A.C., Cryan, J.F., Dalvi, A., Lucki, I., Blendy, J.A., 2002. cAMP response element binding protein is essential for the upregulation of brain derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. Journal of Neuroscience 22, 3262–3268.

Crino, P.B., Eberwine, J., 1996. Molecular characterization of the dendritic growth cone, regulated mRNA transport and local protein synthesis. Neuron 17, 1173–1187.

Gall, C., Lauterborn, J., Guthrie, K., 1995. In situ hybridization: a sensitive measure of activity-dependent changes in neuronal gene expression. In: Stumpf, W.E. (Ed.), Autoradiography and Correlative Imaging. Academic Press, pp. 379–399.

Heersen, H.M., Segal, R.A., 2002. Location, location, location: a spatial view of neurotrophin signal transduction. Trends in Neurosciences 25, 160–166.

Hutter, P., Johansson, M., Saria, A., Humpel, C., 1996. Acute and chronic noradrenergic regulation of neurotrophin messenger RNA expression in rat hippocampus: evidence from lesions and organotypic cultures. Neuroscience 70, 15–29.

Kafitz, K.W., Rose, C.R., Thoenen, H., Konnerth, A., 1999. Neurotrophin-evoked rapid excitation through TrkB receptors. Nature 401, 918–921.

Kokaia, Z., Metsis, M., Kokaia, M., Bengzon, J., Elmer, E., Smith, M.L., Timmusk, T., Siesjo, B.K., Persson, H., Lindvall, O., 1994. Brain insults in rats induce increased expression of the BDNF gene through differential use of multiple promoters. European Journal of Neuroscience 6, 587–596.

Lauterborn, J.C., Poulsen, F.R., Stinis, C.T., Isackson, P.J., Gall, C.M., 1998. Transcript-specific effects of adrenalectomy on seizure-induced BDNF expression in rat hippocampus. Molecular Brain Research 55, 81–91.

Lauterborn, J.C., Rivera, S., Stinis, C.T., Hayes, V.Y., Isackson, P.J., Gall, C.M., 1996. Differential effects of protein synthesis inhibition on the activity-dependent expression of BDNF transcripts: evidence for immediate-early gene responses from specific promoters. Journal of Neuroscience 16, 7428–7436.

Lindefors, N., Brodin, E., Metsis, M., 1995. Spatiotemporal selective effects on brain-derived neurotrophic factor and trkB messenger RNA in rat hippocampus by electroconvulsive shock. Neuroscience 65, 661–670.

Lom, B., Cohen-Cory, S., 1999. Brain-derived neurotrophic factor differentially regulates retinal ganglion cell dendritic and axonal arborization in vivo. Journal of Neuroscience 19, 9928–9938.

Malberg, J.E., Eisch, A.J., Nestler, E.J., Duman, R.S., 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. Journal of Neuroscience 20, 9104–9110.

Metsis, M., Timmusk, T., Arenas, E., Persson, H., 1993. Differential usage of multiple brain-derived neurotrophic factor promoters in the rat brain following neuronal activation. Proceedings of the National Academy of Sciences 90, 8802–8806.

Miro, X., Perez-Torres, S., Artigas, F., Puigdomenech, P., Palacios, J.M., Mengod, G., 2002. Regulation of cAMP phosphodiesterase mRNAs expression in rat brain by acute and chronic fluoxetine treatment. An in situ hybridization study. Neuropharmacology 43, 1148–1157.

Morinobu, S., Strausbaugh, H., Terwilliger, R., Duman, R.S., 1997. Regulation of c-Fos and NGF1-A by antidepressant treatments. Synapse 25, 313–320.

Nakayama, M., Gahara, Y., Kitamure, T., Ohara, O., 1994. Distinctive four promoters collectively direct expression of brain-derived neurotrophic factor gene. Molecular Brain Research 21, 206–218.

Nibuya, M., Morinobu, S., Duman, R.S., 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, E.J., Duman, R.S., 1996. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. Journal of Neuroscience 16, 2365–2372.

Norrholm, S.D., Ouimet, C.C., 2001. Altered dendritic spine density in animal models of depression and in response to antidepressant treatment. Synapse 42, 151–163.

Oliff, H.S., Berchtold, N.C., Isackson, P., Cotman, C.W., 1998. Exercise-induced regulation of BDNF transcripts in the rat hippocampus. Molecular Brain Research 61, 147–153.

Pencea, V., Bingaman, K.D., Wiegand, S.J., Luskin, M.B., 2001.

Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. Journal of Neuroscience 21, 6706–6717.

Righi, M., Tongiorgi, E., Cattaneo, A., 2000. Brain-derived neurotrophic factor (BDNF) induces dendritic targeting of BDNF and tyrosine kinase B mRNAs in hippocampal neurons through a phospatidylinositol-3-kinase-dependent pathway. Journal of Neuroscience 20, 3165–3174.

Russo-Neustadt, A.A., Beard, R.C., Huang, Y.M., Cotman, C.W., 2000. Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. Neuroscience 101, 305–312.

Shirayama, Y., Chen, A.C., Nakagawa, S., Russell, D.S., Duman, R.S., 2002. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. Journal of Neuroscience 22, 3251–3261.

Siuciak, J.A., Lewis, D.R., Wiegand, S.J., Lindsay, R.M., 1997. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacology, Biochemistry and Behaviour 56, 131–137.

Takahashi, M., Terwilliger, R., Lane, C., Mezes, P.S., Conti, M., Duman, R.S., 1999. Chronic antidepressant administration increases the expression of cAMP-specific phosphodiesterase 4A and 4B isoforms. Journal of Neuroscience 19, 610–618.

Tao, X., Finkbeiner, S., Arnold, D.B., Shaywitz, A.J., Greenberg, M.E., 1998. Ca^2 influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron 20, 709–726.

Thoenen, H., 1995. Neurotrophins and neuronal plasticity. Science 270, 593–598.

Thome, J., Sakai, N., Shin, K., Steffen, C., Zhang, Y.J., Impey, S., Storm, D., Duman, R.S., 2000. cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. Journal of Neuroscience 20, 4030–4036.

Timmusk, T., Palm, K., Metsis, M., Reintam, T., Paalme, V., Saarma, M., Persson, H., 1993. Multiple promoters direct the tissue-specific expression of the rat BDNF gene. Neuron 10, 475–489.

Vaidya, V.A., Siuciak, J.A., Du, F., Duman, R.S., 1999. Hippocampal mossy fiber sprouting induced by chronic electroconvulsive seizures. Neuroscience 89, 157–166.

Vaidya, V.A., Duman, R.S., 2001. Depression-emerging insights from neurobiology. British Medical Bulletin 57, 61–79.

Wong, M.L., Licinio, J., 2001. Research and treatment approaches to depression. Nature Reviews Neuroscience 2, 343–351.

Zetterstrom, T.S., Pei, Q., Madhav, T.R., Coppell, A.L., Lewis, L., Grahame-Smith, D.G., 1999. Manipulations of brain 5-HT levels affect gene expression for BDNF in rat brain. Neuropharmacology 38, 1063–1073.