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Short communication

Expression of brain-derived neurotrophic factor is not modulated by chronic mild stress in the rat hippocampus and amygdala

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Abstract:
Accumulating evidence supports a role for brain-derived neurotrophic factor (BDNF) in depression. However, most of these studies have been performed in animal models that have a low face validity with regard to the human disease. Here, we examined the regulation of BDNF expression in the hippocampus and amygdala of rats subjected to the chronic mild stress (CMS) model of depression, a paradigm that induces anhedonia, a core symptom of depression. We found that exposure of rats to the CMS paradigm did not modulate BDNF mRNA expression in the hippocampus and amygdala. In addition, chronic administration of imipramine, which reversed CMS-induced anhedonia, did not alter BDNF mRNA expression in these limbic structures.

Key words:
chronic mild stress, BDNF, depression, anhedonia, imipramine

Abbreviations: BDNF – brain-derived neurotrophic factor, CMS – chronic mild stress, DG – dentate gyrus

Introduction

Depression is a recurrent and potentially life-threatening mental illness that affects hundreds of millions of people worldwide. A triad of clinical symptoms characterize depression: low or depressed mood, anhedonia, and low energy or fatigue [19]. Other symptoms, such as sleep and psychomotor disturbances, pessimism, guilty feelings, low self-esteem, suicidal tendencies, and food-intake and body-weight dysregulation, are also often present [19]. Over the past years, studies have revealed that neuronal atrophy and cell death occur in the brains of depressed patients [9]. In particular, a reduction in the hippocampal volume has

been reported in patients with depression [9]. Positron emission tomography studies have shown multiple abnormalities of regional cerebral blood flow and glucose metabolism in the limbic and prefrontal cortical structures in individuals with major depressive disorder. In particular, regional cerebral blood flow and glucose metabolism are increased in the amygdala of unmedicated subjects with familial depressive disorder, relative to healthy controls [9]. Interestingly, the elevation of cerebral blood flow and glucose metabolism in the amygdala of subjects with major depressive disorder is positively correlated with depression severity [9].

Although the neurobiological basis of depression remains largely unknown, experiments performed with animal models have led to novel hypotheses regarding how depression may occur. In particular, there is increasing evidence that brain-derived neurotrophic factor (BDNF) is involved in the pathophysiology and treatment of depression [5]. The expression of BDNF is reduced in the rat hippocampus after acute and chronic stress, an important factor in the etiology of depression [5]. Conversely, chronic administration of antidepressants increases BDNF expression in this brain region and prevents stress-induced decreases in BDNF levels [5]. Studies using transgenic mice with decreased BDNF levels or reduced signaling through TrkB, the BDNF receptor, have shown that TrkB activation is required for the behavioral effects induced by antidepressants [15]. Together, these observations provide evidence for a link between BDNF levels, the appearance of depressive symptoms, and their treatment with antidepressants. However, most studies supporting a role for BDNF in depression have been performed in behavioral models of depression that have a low face validity with regard to the human disease, such as the learned helplessness and the forced swim test. Although these animal models of depression have a good predictive value to identify new antidepressant treatments, they have a poor face validity with regard to the human disease as the extent of phenomenological similarities between the model and the disorder is low [18].

To advance our understanding of the role of BDNF in depression, we examined the expression of BDNF in the hippocampus and amygdala of animals exposed to chronic mild stress (CMS) in the presence or absence of the antidepressant imipramine. The CMS model of depression is considered to have a greater face validity than the learned helplessness and the forced swim test [17, 18]. In the CMS paradigm, rats are subjected to a variety of mild stressors presented intermittently for prolonged periods of time (e.g., several weeks). This animal model causes a generalized decrease in responsiveness to rewards, which is comparable to anhedonia, a core symptom of depression [17]. Anhedonia is monitored by a reduction in sucrose consumption, which can be reversed by chronic treatment with a wide variety of antidepressants, such as the tricyclic antidepressant imipramine [16, 17].

Data from this study revealed that exposure of rats to the CMS paradigm did not alter BDNF mRNA expression in the hippocampus and amygdala. We also found that chronic administration of imipramine, which reversed CMS-induced anhedonia, did not modulate BDNF mRNA expression in these limbic regions.

Materials and Methods

Chronic mild stress

Studies were approved by the Bioethical Committee of the Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland.

The CMS procedure was performed according to Papp et al. [13]. Briefly, male Wistar rats (Górzkowska, Warszawa) were trained to consume a 1% sucrose solution. Following the final baseline test, rats were divided in two groups: one subjected to a CMS procedure for a period of seven consecutive weeks and a second left unchallenged (control rats). The weekly stress regime consisted of two periods of food and water deprivation, 45° cage tilt, intermittent illumination, a soiled cage, paired housing, low intensity stroboscopic illumination, and two periods of no stress. Control rats and CMS animals, which were housed in separate rooms, had free access to food and water except for a period (14 h) preceding each sucrose test. Following 2 weeks of stress, both CMS and control animals were divided in subgroups. Animals from the CMS and control groups were injected once daily with vehicle (1 ml/kg, *ip*), or imipramine (10 mg/kg, *ip*) for the subsequent 5 weeks, and sucrose tests were performed once weekly, 24 h after the last drug administration. After 5 weeks of treatment (24 h after the final injection) vehicle-treated control animals (CV),

imipramine-treated control animals (CI), animals subjected to CMS and treated chronically with vehicle (SV), animals subjected to CMS and treated chronically with imipramine (SI), and animals exposed to CMS that did not respond to imipramine administration (non-responders, SIN) were decapitated and processed for *in situ* hybridization. Rats were considered non-responders when, by the end of the 5-weeks of imipramine administration, their sucrose consumption was comparable to that measured following the 2-weeks of stress.

Values are the mean ± SEM of sucrose intake. Sucrose intake data were analyzed by a repeated measures two-way (mixed model) ANOVA test with Bonferroni *post-hoc* test, using the Prism software (GraphPad Software, Inc., La Jolla, USA), $p < 0.05$ was considered statistically significant.

In situ hybridization

Coronal brain sections (16 μm, –2.5 mm to –3.5 mm relative to the bregma) were cut on a cryostat (Leica Microsystems, Switzerland) and thaw-mounted onto glass slides. *In situ* hybridization experiments were performed as previously described [11]. [^{35}S]-labeled antisense and sense riboprobes were generated by *in vitro* transcription from a linearized pGEM-T Easy vector (Promega, Madison, WI, USA) containing a 549 bp BDNF cDNA fragment obtained by reverse transcription and polymerase chain reaction amplification using the following set of primers: (201–219) 5'-ACTCTGGAGAGCGTGAATG-3' and (749–732) 5'-GCTATCCATAGTAAGGC-3' (GenBank accession number X55573). At the end of the experiment, brain sections were exposed to BioMax MR-1 X-ray films (Kodak, Rochester, NY, USA) for 4–7 days at room temperature. Finally, slides were dipped in NTB2 nuclear track emulsion (Kodak, Rochester, NY, USA), exposed in the dark for 4–6 weeks, developed in D19 (Kodak, Rochester, NY, USA), and counterstained with 0.02% blue toluidine. Dark- and bright-field images were captured on a Leica MZ16FA and a Zeiss Axiovision microscope equipped with CCD cameras, respectively. Data from hybridization signals were analyzed by optical densitometry of autoradiographic films using a computerized image analysis system (MCID, Imaging Research, St. Catherine's, Ontario, Canada). Optical density value for each brain region analyzed was normalized to the total optical density value of the section after subtraction of the selected regions. Relative optical density values for each animal were the mean of at least 10 sections for each region. Since no difference in relative optical density values were observed between right and left hemispheres, data for each region from both hemispheres were averaged.

Data are the mean ± SEM of relative optical density values. Statistical analysis of relative optical densities was made using the SAS package (SAS Institute Inc., Cary, NC, USA). The hierarchical ANOVA test was used to analyze differences between groups for each region of interest, $p < 0.05$ was considered statistically significant.

Results

Analysis of sucrose intake revealed that 2 weeks of initial exposure to CMS caused a gradual and significant decrease in the consumption of the sucrose solution compared to control animals, indicating that CMS induces a decrease in sensitivity to reward (Fig. 1). Differences in sucrose intake between control and stressed animals persisted over the course of the experiment (Fig. 1). In control animals, sucrose con-

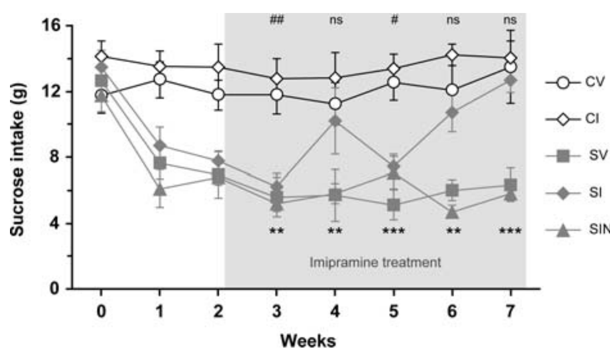

Fig. 1. Effects of chronic mild stress (CMS) and imipramine administration on sucrose consumption. Animals were separated in two groups, one undergoing a CMS protocol and the other one left unchallenged. After 2 weeks of initial exposure to CMS, the stressed group was subdivided in three subgroups: vehicle (SV), imipramine (SI), and non-responders to imipramine (SIN). Unchallenged animals were subdivided in two subgroups: vehicle (CV) and imipramine (CI). Values are the mean ± SEM of sucrose intake (n = 8 rats per experimental condition from two independent CMS experiments). Statistical analysis was shown only for time points during imipramine treatment (weeks 3–7). ** $p < 0.01$, *** $p < 0.001$ between SV and CV sucrose intake values. # $p < 0.05$, ## $p < 0.01$, not significantly different (ns) between SI and CV sucrose intake values. No statistically significant differences were observed between SV and SIN subgroups and between CV and CI animals

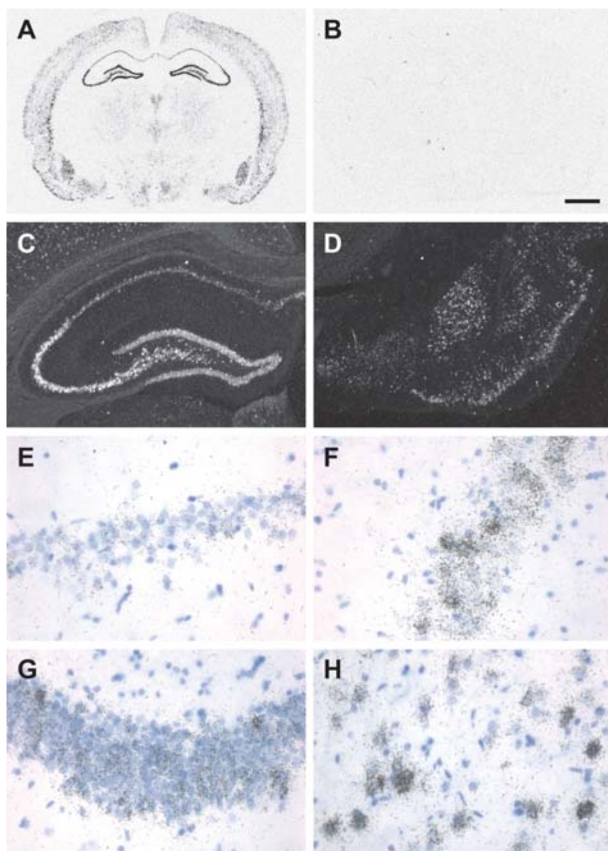

Fig. 2. Distribution of brain-derived neurotrophic factor (BDNF) mRNA expression in the hippocampus and amygdala of control animals. **(A)** Representative autoradiographic film image of BDNF mRNA expression in the brain of vehicle-treated control rats. **(B)** Adjacent section incubated with [^{35}S]-labeled sense riboprobe. **(C)** Representative dark-field photomicrograph showing BDNF mRNA expression in the pyramidal neurons of CA1 and CA3 of Ammon's Horn, as well as in the granule neurons of the dentate gyrus (DG). **(D)** Representative dark-field photomicrograph showing BDNF mRNA expression in the amygdala. **(E–H)** High-magnification bright-field photomicrographs showing the cellular distribution of BDNF mRNA labeling in CA1 (E), CA3 (F), DG (G) and amygdala (H). Scale bar = 1.85 mm in A, B; 0.45 mm in C, D and 20 µm in E, F, G, H

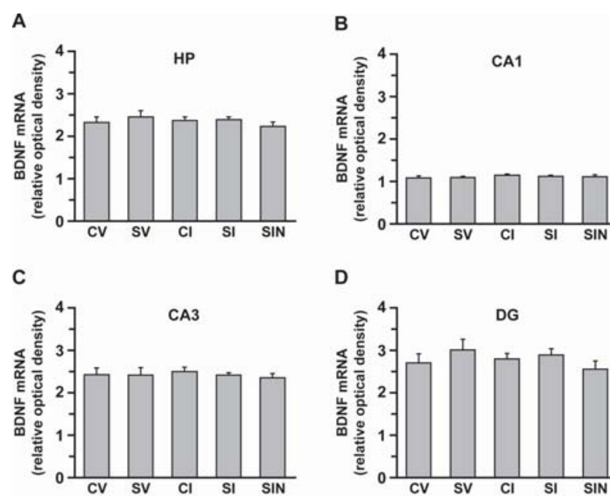

Fig. 3. Chronic mild stress (CMS) and imipramine treatment do not affect brain-derived neurotrophic factor (BDNF) mRNA levels in the rat hippocampus. BDNF mRNA levels in total hippocampus (HP) **(A)**, CA1 **(B)**, CA3 **(C)**, and DG **(D)** of vehicle-treated control animals (CV), of animals subjected to CMS and treated chronically with vehicle (SV), of imipramine-treated control animals (CI), of animals subjected to CMS and treated chronically with imipramine (SI), and of animals exposed to CMS that did not respond to imipramine administration (non-responders, SIN). BDNF mRNA expression was analyzed by *in situ* hybridization combined with optical densitometry. Data are the mean ± SEM of relative optical density values (n = 8 per experimental condition from two independent CMS experiments). Statistical analysis indicated no significant differences in BDNF mRNA levels between treatment groups in any of the hippocampal subfields examined

sumption was not affected by imipramine, while chronic administration of the tricyclic antidepressant to stressed animals reversed the decrease in sucrose intake (Fig. 1). In these animals, sucrose consumption increased from 7.8 ± 0.6 g (n = 8; week 2) to 12.7 ± 0.8 g (n = 8; week 7) after 5 weeks of treatment with imipramine, reaching sucrose intake values that were not significantly different from vehicle-treated control animals (Fig. 1). CMS animals that did not respond to imipramine administration (non-responders) did not show significant differences in sucrose intake compared to stressed animals treated with vehicle (Fig. 1).

The effects of CMS and imipramine treatment on BDNF mRNA expression were analyzed in different subfields of the hippocampus, as well as in the amygdala. *In situ* hybridization analysis showed that BDNF mRNA was expressed in all cytoarchitectural divisions of the hippocampus (Fig. 2A). Thus, the hybridization signal was detected in the pyramidal layers of all CA subfields of the hippocampus and in the granule cell layer of the dentate gyrus (DG, Fig. 2A, C), which is consistent with previous observations [4]. Interestingly, the hybridization signal was not evenly distributed; the strongest hybridization signal was observed in the DG and CA3 area, whereas BDNF mRNA labeling in the CA1 subfield was modest. Bright-field photomicrographs revealed that in the hippocampal CA1 area, most of the cells were lightly labeled, indicative of a low BDNF mRNA content, while cells in the CA3 area and DG were moderately or heavily labeled (Fig. 2E–G).

In the amygdala, a moderate expression of BDNF mRNA was observed in the basolateral nucleus,

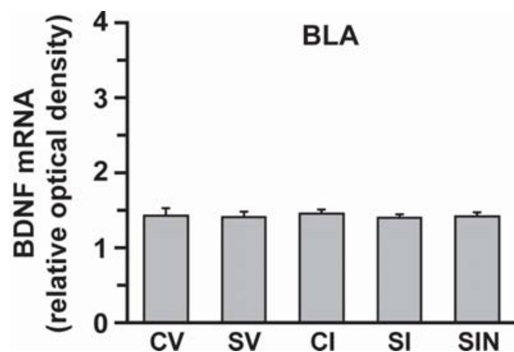

Fig. 4. Chronic mild stress (CMS) and imipramine treatment do not alter brain-derived neurotrophic factor (BDNF) mRNA levels in the basolateral amygdala. BDNF mRNA levels in the basolateral amygdala (BLA) of vehicle-treated control animals (CV), of animals exposed to CMS and treated chronically with vehicle (SV), of imipramine-treated control animals (CI), of animals subjected to CMS and treated chronically with imipramine (SI), and of animals exposed to CMS that did not respond to imipramine administration (non-responders, SIN). BDNF mRNA expression was analyzed by *in situ* hybridization combined with optical densitometry. Data are the mean ± SEM of relative optical density values (n = 8 per experimental condition from two independent CMS experiments). Statistical analysis indicated no significant differences in BDNF mRNA levels between treatment groups

whereas cells containing BDNF mRNA were absent from the central nucleus (Fig. 2D). High-magnification bright-field photomicrographs showed that the cellular distribution of BDNF mRNA in the basolateral nucleus of the amygdala was patchy with some cells displaying a high density of silver grains, while others were completely devoid of labeling (Fig. 2H).

BDNF mRNA expression was examined in the hippocampal subfields CA1, CA3, and DG of control animals treated with vehicle (CV) or imipramine (CI), of animals subjected to CMS and treated with vehicle (SV) or imipramine (SI), and of animals exposed to CMS that did not respond to imipramine administration (SIN). Optical densitometry of autoradiographic films showed no significant difference in BDNF mRNA expression between treatment groups in any measured region of the Ammon's horn or in the DG (Fig. 3). These results indicate that BDNF mRNA expression in the rat hippocampus is not modulated by CMS or imipramine administration under the experimental conditions that induce anhedonia.

Although several brain regions have been implicated in the pathophysiology of depression, the amygdala is of particular interest. Imaging studies in depressed patients have shown abnormal elevations of resting cerebral blood flow and glucose metabolism in this limbic structure, as well as reduced core amygdala nuclei volume [9]. This led us to examine whether BDNF mRNA expression was altered by CMS and imipramine in the basolateral amygdala, a nucleus enriched in BDNF mRNA that is critical for the formation of emotional memories. Similarly to data observed in the hippocampus, no significant difference in BDNF mRNA levels between treatment groups was found in the basolateral amygdala (Fig. 4), indicating that BDNF mRNA expression is not modulated by CMS-induced anhedonia or chronic administration of imipramine.

Discussion

Data from the current study demonstrate that exposure of rats to the CMS paradigm does not alter BDNF mRNA expression in the hippocampus and in the basolateral amygdala. These results indicate that BDNF mRNA levels are not modulated in these limbic brain structures during experimental conditions that cause anhedonia, a core symptom of depression. This study also provides evidence that chronic administration of the tricyclic antidepressant imipramine, which reverses CMS-induced anhedonia, does not regulate BDNF mRNA expression in the hippocampus and in the basolateral amygdala.

The original hypothesis regarding the role of BDNF in depression was based on different observations showing that the physical and psychological stress that induces depression-like behavior in rodents modulates endogenous BDNF expression. The first demonstration of a relationship between stress and BDNF expression was established using an immobilization stress paradigm involving the physical restraint of rats. These studies revealed that immobilization stress caused significant decreases in BDNF mRNA levels in the DG, CA1, and CA3 pyramidal cell layers [5]. These data were further confirmed using other stress paradigms. For instance, hippocampal BDNF levels were found to be reduced after the learned helplessness, the forced swim test, re-exposing rats to cues previously associated with footshock, social defeat, or social isolation [6, 7, 14]. In contrast to the effects of stress, a range of pharmacological antidepressants, including monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, noradrenaline reuptake in-

hibitors, and tricyclic antidepressants, were shown to increase BDNF levels in the hippocampus [6].

Although these findings support a role for BDNF in the etiology and treatment of depression, it is important to emphasize that the animal models used in these studies have a limited face validity with regard to the human disease. Indeed, the extent of phenomenological similarities between these animal models and the human disorder is low. In contrast, the CMS model of depression has a unique combination of face validity (resemblance to the human symptoms), predictive validity (expected responses to treatments that are effective in the human disease), and construct validity (similarity to the underlying cause of the disease) [16, 18]. With regard to the face validity of the CMS model, it has been shown that in addition to anhedonia, the CMS paradigm induces the appearance of other behavioral phenomena that reflect symptoms of depression, such as decreases in sexual and aggressive behaviors, as well as reduced grooming and REM sleep latency [16]. Although there is no standard CMS procedure, they all use a variety of mild stressors, scheduled in a relatively unpredictable sequence over a period of several weeks, and they largely avoid severe stressors, such as footshock, extremes of temperature, and prolonged food/water deprivation [16].

In this study, analysis of BDNF mRNA expression in different subfields of the hippocampus and in the basolateral amygdala of adult rats subjected to CMS did not reveal significant changes in BDNF mRNA levels (Fig. 3, 4). These data are in agreement with previous studies showing that exposure of rats to CMS does not change BDNF mRNA levels in the DG [8] and subfield CA3 [2] of the hippocampus.

Interestingly, regulation of BDNF mRNA expression by CMS differs from studies in which the learned helplessness and the forced swim test were used as behavioral models of depression. In the learned helplessness paradigm, rats subjected to inescapable footshock exhibited decreased BDNF levels in the hippocampus, while chronic administration of imipramine reversed the downregulation of BDNF expression in this limbic region [7]. Similarly to the regulation of BDNF expression in the learned helplessness paradigm, exposure of rats to the forced swim test caused a significant reduction of BDNF mRNA levels in several hippocampal subfields including CA1, CA3, and DG, as revealed by *in situ* hybridization, while chronic administration of the monoamine oxidase inhibitor tranylcypromine reversed the stress-induced decline in the levels of BDNF mRNA [14]. Although data presented in this study contrast with these findings, it is important to point out that the nature and severity of the stressors used in the CMS model of depression markedly differ from those employed in the learned helplessness and the forced swim test paradigms. The learned helplessness paradigm uses extremely severe stressors and it remains unclear to what extent learned helplessness is a model of post-traumatic stress disorder rather than depression [6, 18]. There is ongoing controversy about the forced swim test as to whether this animal model induces depression-like symptoms or whether it is merely a testing protocol for detecting agents with antidepressant-like activity [12]. In marked contrast, the CMS procedure uses a variety of moderate stressors and avoids severe stressors [16].

The severity of the stressors used in the behavioral paradigms have been suggested to play a critical role in modeling depression [1]. A chronic mild stressor paradigm similar to that used in the CMS model of depression and one that relies on fairly intense acute stressor application, like in the learned helplessness paradigm, may not induce identical syndromes, and hence may involve different underlying mechanisms and may be differentially influenced by antidepressant treatment.

The impact of stressors is dependent upon the characteristics of the stressor, such as severity, chronicity, and predictability [1]. Interestingly, regulation of BDNF expression by stress in the adult hippocampus is dependent on the nature of the stressor, its intensity, duration, and frequency [10]. In this regard, recent studies have shown that acute and chronic immobilization stress decreases BDNF mRNA levels, while chronic unpredictable stress does not alter BDNF mRNA expression in the adult hippocampus, indicating that regulation of BDNF expression is stressor specific [10].

In conclusion, regulation of BDNF expression in behavioral models of depression is undoubtedly more complex than revealed by previous work. Indeed, data presented in this study and others [6] suggest that the nature and severity of the stressors used to model depression are critical for the regulation of BDNF expression. Finally, it has been recently proposed that depression and antidepressant effects may not be simply correlated with the levels of BDNF in the brain but that BDNF may modify, in an activity-dependent manner, the structure of neuronal networks, the func-

tion of which would determine whether changes in plasticity produce a depression- or antidepressant-like behavioral response in experimental animals [3].

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