Activation of AMPA Receptors Mediates the Antidepressant Action of Deep Brain Stimulation of the Infralimbic Prefrontal Cortex

Laura Jiménez-Sánchez1,2, Anna Castañé1,2, Laura Pérez-Caballero2,3, Marc Grifoll-Escoda1, Xavier López-Gil1,2,4, Leticia Campa1,2, Mireia Galofré1,2, Esther Berrocoso2,3 and Albert Adell1,2,5

1Department of Neurochemistry and Neuropharmacology, Instituto de Investigaciones Biomédicas de Barcelona, CSIC, IDIBAPS, Barcelona 08036, Spain, 2Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Spain, 3Neuropsychopharmacology and Psychobiology Research Group, Department of Psychology, University of Cadiz, Cadiz 11510, Spain, 4Current address: Experimental 7T MRI Unit, Institut d’Investigacions Biomèdiques August Pi i Sunyer, Barcelona 08036, Spain and 5Current address: Instituto de Biomedicina y Biotecnología de Cantabria, IBBTEC (CSIC, Universidad de Cantabria), Santander 39011, Spain

Address correspondence to Dr Albert Adell. Email: albert.adell@unican.es

Abstract

Although deep brain stimulation (DBS) has been used with success in treatment-resistant depression, little is known about its mechanism of action. We examined the antidepressant-like activity of short (1 h) DBS applied to the infralimbic prefrontal cortex in the forced swim test (FST) and the novelty-suppressed feeding test (NSFT). We also used in vivo microdialysis to evaluate the release of glutamate, γ-aminobutyric acid, serotonin, dopamine, and noradrenaline in the prefrontal cortex and c-Fos immunohistochemistry to determine the brain regions activated by DBS. One hour of DBS of the infralimbic prefrontal cortex has antidepressant-like effects in FST and NSFT, and increases prefrontal efflux of glutamate, which would activate AMPA receptors (AMPARs). This effect is specific of the infralimbic area since it is not observed after DBS of the prelimbic subregion. The activation of prefrontal AMPARs would result in a stimulation of prefrontal output to the brainstem, thus increasing serotonin, dopamine, and noradrenaline in the prefrontal cortex. Further, the activation of prefrontal AMPARs is necessary and sufficient condition for the antidepressant response of 1 h DBS.

Key words: c-Fos, dorsal raphe nucleus, glutamate, microdialysis, serotonin

Introduction

Depression is the most common condition among neuropsychiatric disorders. It is likely that many brain regions mediate the array of depressive symptoms: reward and motivation (nucleus accumbens), fear and anxiety (amygdala), depressed mood (limbic system), cognitive impairment (prefrontal cortex), and vegetative symptoms, such as changes in hormonal secretion, appetite, sexual drive, sleep (hypothalamus). High-frequency deep brain stimulation (DBS) has emerged as a new experimental therapy for treatment-resistant depression. Neuroimaging
studies have demonstrated that the subgenual cingulate gyrus (SCG) is overactive in depression and its activity normalized after effective antidepressant treatment with fluoxetine (Mayberg et al. 2000), electroconvulsive therapy (Nobler et al. 2001), repetitive transcranial magnetic stimulation (Mottaghy et al. 2002), ablative surgery (Dougerty et al. 2003), and DBS (Mayberg et al. 2005). This suppression of activity of the stimulated area led to postulate that the antidepressant action of DBS was caused by a functional lesion induced by high-frequency stimulation. Nevertheless, recent work has suggested that DBS produces an inhibition restricted to the stimulated area associated with an activation of the nearby axons (McIntyre et al. 2004). Indeed, a clear activation of distant brain areas by DBS has been demonstrated (Schuckermair et al. 2013). Despite its clinical efficacy, the mechanism of action of DBS in vivo remains to be elucidated. There is general agreement, however, that DBS impacts on circuits implicated in the regulation of mood that are impaired in depression (Lozano and Lipsman 2013).

For more than 50 years now, pharmacotherapy for depression has relied on the action of antidepressants on monoaminergic systems in the brain and, indeed, more than 90% of marketed drugs target these systems. These neurotransmitter systems are extensively distributed throughout the network of limbic, striatal, and prefrontal cortical neuronal circuits thought to support the behavioral and visceral manifestations of mood disorders. Indeed, the medial prefrontal cortex (mPFC) as a whole has been traditionally implicated in attentional processes (noradrenaline), working memory (dopamine), and behavioral flexibility (serotonin, 5-hydroxytryptamine [5-HT]). In the last decade, however, interest on new antidepressant drugs has turned from monoamines to glutamate (Sanacora et al. 2008). Indeed, neuroimaging (Auer et al. 2000; Sanacora et al. 2004; Hasler et al. 2007; Yüksel and Öngür 2010) and postmortem (Hashimoto et al. 2007; Feyissa et al. 2010) studies have reported abnormalities of glutamatergic systems in depression, and a glutamatergic drug such as ketamine is a clinically effective, fast-acting antidepressant treatment under experimental clinical examination (Berman et al. 2000; Zarate et al. 2006; aan het Rot et al. 2010). Interestingly, the antidepressant-like behavior of ketamine is dependent on the activation of α-amino-3-hydroxy-5-methyl-4-isoxazolopyridinonic acid (AMPA) receptors (AMPARs) (Maeng et al. 2008; Autry et al. 2011), and a recent study also showed that the sole systemic administration of AMPA produces antidepressant-like effects in rats (Akinfresayev and Tizabi 2013).

In the rodent brain, the infralimbic (IL) and the ventral part of the prelimbic (PL) portion of the mPFC, as a whole, is considered to be the rodent homolog of SCG (Gabbott et al. 2003). Thereafter, we will designate this subregion as IL in contrast to the PL, which will be used for the more dorsal part of the mPFC. The IL is an important hub, connected to diverse regions implicated in the pathophysiology of depression, such as hippocampus, amygdala, nucleus accumbens, and brainstem monoaminergic nuclei (Heidbreder and Groenewegen 2003). The first purpose of the present work was to examine the behavioral response to short-term (1 h) DBS in rodent models relevant to depression—the forced swim test (FST)—and anxiety—the novelty-suppressed feeding test (NSFT). It is of note that the FST is sensitive to a single dose of most prescribed antidepressant drugs, whereas only chronic dosage is effective in the NSFT (Bodnoff et al. 1988). The relationship between the behavioral actions of DBS and changes in the in vivo outflow of glutamate, noradrenaline, dopamine, and 5-HT in the mPFC were examined as well. The neuronal activation of different brain areas after 1 h DBS was assessed by c-Fos immunohistochemistry.

### Materials and Methods

#### Subjects and Drugs

Male Wistar rats (Charles River Laboratories, Cerdanyola del Vallès, Spain) weighing 280–350 g were used. Food and water were always freely available. All experimental procedures were in strict accordance with national (Royal Decree 53/2013) and European legislation (Directive 2010/63/EU of the European Parliament and of the Council, 22 September 2010, on the protection of animals used for scientific purposes), and were approved by the Institutional Animal Care and Use Committee of the University of Barcelona.

Glutamate, 5-HT, dopamine hydrochloride, noradrenaline, γ-aminobutyric acid (GABA), p-chlorophenylalanine (pCPA), and o-phthalaldehyde (OPA) reagent (containing 1 mg OPA per ml solution with 2-mercaptopethanol as the sulfydryl moiety) were purchased from Sigma-Aldrich (Tres Cantos, Spain). Citalopram hydrobromide, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydropenzo[1]quinoxaline-7-sulfonamide disodium salt (NBQX), (S)-AMPA, and nomifensine maleate were purchased from Tocris (Bristol, UK). NBQX (300 μm), and (S)-AMPA (100 μm) were dissolved in artificial cerebrospinal fluid (aCSF, see below for composition) for intracerebral administration. NBQX (10 mg/kg) and pCPA (150 mg/kg) were dissolved in saline for intraperitoneal (i.p.) administration. Appropriate vehicles were administered as the respective control groups.

#### Deep Brain Stimulation

Bipolar stimulating electrodes were implanted bilaterally under pentobarbital anesthesia (60 mg/kg, i.p.) in the IL mPFC (AP +3.2 mm, L ±0.6 mm, DV −5.4 mm from bregma) or in the PL mPFC (AP +3.2 mm, L ±0.6 mm, DV −3.3 mm from bregma), according to reference atlas (Paxinos and Watson 2005). They consisted of 2 stainless steel enamel-coated wires (California Fine Wire, Grover Beach, CA, USA) with a diameter of 150 μm and a tip separation of ~100 μm and in vitro impedances of 10–30 KΩ. When the release of transmitters in the mPFC was examined, one electrode was attached to the shaft of the dialysis probe whereas the other one was implanted alone in the contralateral mPFC. In all other experiments, only the electrodes were implanted bilaterally in the mPFC. Stimulation lasted for 1 h and its parameters were: frequency, 130 Hz; current intensity, 200 μA, and pulse width, 90 μs. Current was delivered with a CS-20 Stimulator (Cibertecc, Madrid, Spain) attached to an overhead electrical swivel (Plastics One, Inc., Roanoke, VA, USA). In control (sham) groups, rats had the 2 electrodes implanted, but no current was delivered.

#### Microdialysis Procedures

Concentric dialysis probes with a 4-mm membrane length were implanted under sodium pentobarbital anesthesia (60 mg/kg, i.p.) in the IL mPFC (AP +3.2 mm, L ±0.6 mm, DV −6.0 mm from bregma), according to reference atlas (Paxinos and Watson 2005). When a dual probe microdialysis approach was used, the second probe was implanted in the dorsal raphe nucleus (DR) (AP −7.8 mm, L-3.1 mm, DV-7.8 mm, with an angle of 30° to avoid the aqueduct), according to reference atlas (Paxinos and Watson 2005). Microdialysis experiments were conducted 48 h after surgery in freely moving rats by continuously perfusing probes with aCSF (125 mM NaCl, 2.5 mM KCl, 1.26 mM CaCl2, 1.18 mM MgCl2, and 1 μM citalopram) at a rate of 1.5 μL/min. Dialysate samples of 30 μL were collected every 20 min. When
dopamine and noradrenaline were determined, aCSF contained 10 μM nomifensine instead of citalopram, and dialysate fractions were collected on microvials containing 5 μL of 10 mM perchloric acid and rapidly injected into the high performance liquid chromatography (HPLC) system. At the completion of experiments, rats were given an overdose of sodium pentobarbital and the brains were then rapidly removed, frozen on dry ice, and stored at −80°C. Tissue sections were cut using a cryostat (HM500 OM; Microm, Walldorf, Germany) and probe and electrode placements were confirmed in histological sections stained with cresyl violet dye (Sigma-Aldrich). Experimental data from misplaced probes or electrodes were discarded. 5-HT, dopamine, and noradrenaline were determined with HPLC coupled to electrochemical detection whereas glutamate and GABA were determined with HPLC coupled to fluorimetric detection (López-Gil et al. 2007; Masana et al. 2012).

c-Fos Immunostaining

C-Fos expression is considered to be a marker of neuronal activity (Dragunow and Faull 1989). Immunohistochemistry protocol used was as described previously (Zuo et al. 2009). In order to mimic the microdialysis setting, a group of rats were implanted bilaterally with 2 electrodes in PL or IL PFC. Forty-eight hour later, 1 h DBS (DBS on) or no current (DBS off) was delivered. Two hours after DBS offset rats were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and then perfused transcardially with cold 0.9% NaCl followed by 10% buffered formalin (Sigma-Aldrich). Each brain was removed immediately, post-fixed in the same fixative for 24 h at 4°C, and then cut on a vibratome in the coronal plane at room temperature (RT) in 3% H2O2 to inactivate endogenous peroxidase. Thereafter, sections were washed twice in 0.01 M phosphate-buffered saline (PBS) containing 0.2% Triton X-100. After blocking with 5% bovine serum albumina (BSA) for 1 h at RT, sections were incubated overnight at 4°C with rabbit polyclonal antibody, sections were washed 3 times in PBS containing 0.2% Triton X-100, 0.2% gelatin and 5% BSA. Following incubation in the primary antibody, sections were washed 3 times in PBS containing 0.2% Triton X-100 and incubated with biotinylated goat anti-rabbit immunoglobulin G (fGg) antiserum (1:200, Vector Laboratories, Burlingame, CA, USA), for 1 h at RT. Sections were then washed with PBS containing 0.2% Triton X-100, incubated for 1 h with avidin–biotin peroxidase complex (1:1000; Vectastain ABC kit, Vector Laboratories), washed in 0.01 M PBS, and finally revealed with diaminobenzidine for 10 min. Sections were then slide-mounted onto gelatin-coated slides. The number of c-Fos-positive cells was measured in triplicate. The procedure also included negative controls with omission of the primary antibody, which did not show any immunoreactions. c-Fos-positive nuclei were counted relative to a threshold based on staining density, which was established by visual observation blinded to treatment. Brain sections were examined in an Olympus BX51 Stereo Microscope (Japan). Cell counting was performed using Visiopharm Integrator System software for stereological analysis (Visiopharm A/S, Hoersholm, Denmark).

cortical transection. This was performed as described previously (Diaz-Mataix et al. 2005) under pentobarbital anesthesia (60 mg/kg, i.p.), using a fine metal needle (0.6 mm diameter). The needle was first positioned at AF +2.0, DV −6.8, and L +0.8 mm from bregma and moved stereotactically (Paxinos and Watson 2005) to L +4.8 in the left hemisphere. Then it was placed in the right hemisphere with an angle of 20° (to avoid damage of the sagittal sinus), at coordinates AF +2.0, DV −6.8, and L −1.2 mm and moved from −1.2 to −4.8 mm. Cortical transection was carried out immediately before probe implantation. Microdialysis experiments were performed 2 days after surgery.

Forced Swim Test

Rats were handled daily for 1 week before FST. On day 1 (pretest, 24 h after electrode implantation), rats were placed in a clear plexiglas cylinder (46 cm height, 20 cm diameter) filled with 24 ± 1°C water to a height of 30 cm, for 15 min. After this pretest, animals were returned to their home cages and dried under a lamp for 30 min. The test was conducted 24 h after the pretest session in the same cylinder for 5 min. Immediately before this test session, rats were given 1 h DBS (DBS on) or no current (DBS off) in their home cages. The 5-min test session was divided into 5-s epochs. At the end of each epoch, the predominant behavior was rated as immobility, climbing, and swimming by an experimenter blind to the treatment.

Locomotor Activity Test

To check for unspeciﬁc changes in gross activity that would mask FST observations, locomotor activity was measured in an open ﬁeld arena (100 cm × 100 cm × 40 cm) with black plastic walls dimly lighted. Locomotor activity was recorded during 15 min by a videocamera connected to a computer (Videotrack).

Novelty-Suppressed Feeding Test

This test assesses the conﬂict behavior of the rat’s aversion to eating in a novel environment. Notably, this test is uniquely sensitive to chronic versus acute antidepressant treatment. Animals were habituated to sweet pellets during 1 week. Twenty-four hours before the experiment, rats were deprived of food, but allowed to drink water ad libitum. Immediately after 1 h of IL DBS, animals were placed in an open ﬁeld arena (100 cm × 100 cm × 40 cm) highly illuminated (800–1000 lx) with food in the center. Latency to consume the food was measured blindly (simply sniffing or touching the food was not considered).

Statistical Analysis

Data are expressed as mean ± SEM. In general, statistical analysis of normally distributed datasets consisted of two-way analysis of variance (ANOVA), followed by Newman–Keuls post hoc tests. Comparisons between 2 groups were assessed by two-tailed Student’s t-test. In all cases, the level of signiﬁcance was set at P < 0.05.

Results

Antidepressant-like Effects of IL DBS: Comparison with PL DBS

The stimulation of IL (130 Hz, 200 μA, and 90 μs) for 1 h (Fig. 1A,B) induced an antidepressant-like response in the FST carried out immediately thereafter, measured as decreased immobility and
increased climbing (Fig. 1C), and decreased the latency to feed in the NSFT (Fig. 1D) also carried out immediately thereafter. These effects were achieved without any concomitant alteration of general locomotor activity (Supplementary Fig. 1A). Further, IL DBS produced ~150% increase in the release of glutamate, 5-HT, dopamine, and noradrenaline in the mPFC (Fig. 1E–H) that remained elevated after DBS cessation. In contrast, IL DBS did not alter GABA level (Fig. 1I). When DBS was applied to the more dorsal part of
the mPFC, namely the PL subregion (Fig. 2A,B), no behavioral change was observed in the FST (Fig. 2C). Interestingly, PL DBS resulted in clear decreases in cortical glutamate, dopamine and noradrenaline effluxes, with no change in 5-HT (Fig. 2D–G).

IL and PL DBS Activate Prefrontal Output
To study the effects of IL and PL DBS on network changes, we next examined c-Fos immunolabeling throughout the rat brain as a measure of neuronal activation. Although a small blank area was observed adjacent to the electrode tip (see Figs 1B and 2B) reminiscent of a functional inactivation of this small area, the overall effects of DBS were suggestive of a downstream activation of several cortical and subcortical brain areas (Fig. 3A,B). In parallel with clinical observations, the antidepressant-like actions of DBS were seen only when stimulation was circumscribed to IL. Interestingly, the DR, ventral tegmental area (VTA) and the locus coeruleus (LC) did not show any c-Fos expression 2 h after IL DBS (data not shown). Although DBS of the IL and PL appeared to produce similar qualitative changes of c-Fos induction in several brain areas, marked quantitative effects were encountered depending on the area stimulated (see Fig. 3C).

Figure 2. Effects of 1 h DBS (130 Hz, 200 µA, 90 µs) in the PL. (A) Scheme and cresyl violet staining of a section of a rat brain showing the implantation of bilateral electrodes and unilateral dialysis probe. (B) c-Fos immunostaining of the electrode tip area. (C) PL DBS did not produce any behavioral change in the FST (Student’s t-test). (D) PL DBS decreased the mPFC efflux of glutamate. (E) PL DBS did not change the mPFC efflux of 5-HT. (F) PL DBS decreased the mPFC efflux of dopamine. (G) PL DBS decreased the mPFC efflux of noradrenaline. Results are expressed as mean ± SEM of n = 5–9 rats per group (Newman-Keuls test following significant two-way repeated-measures ANOVA, *P < 0.05, **P < 0.001 in D, F, and G). Scale bar in B, 200 µm.
To gain further insight into the changes in cortico-raphe projection, we implanted 2 electrodes in the IL, as above, and a dialysis probe in the DR (Fig. 5A,B). Our results showed that IL DBS increased the efflux of glutamate in the DR (Fig. 5A) without altering that of 5-HT (Fig. 5B). In another group of rats, we next performed a dual probe experiment in which 2 probes were implanted, one in the mPFC and the other in the DR (Fig. 5C). Consistently with our previous experiment, the administration of increasing concentrations of glutamate in the DR did not alter the local efflux of 5-HT (Fig. 5D), but increased the dialysate concentrations of both glutamate (Fig. 5E) and 5-HT (Fig. 5F) in the mPFC.

Role of AMPARs in the Antidepressant Effects of IL DBS

As depicted in Figure 6A, both the systemic (10 mg/kg, 5 min before IL DBS onset) and intra-mPFC (300 µM in each hemisphere, starting 5 min before and lasting 1 more h during IL DBS) administration of the AMPAR antagonist, NBQX, suppressed the antidepressant-like action of IL DBS in the FST carried out immediately thereafter. In addition, NBQX blocked the IL DBS-induced increase in prefrontal 5-HT—albeit only after DBS cessation (Fig. 6B)—and glutamate (Fig. 6C) release. To gain further insight in the antidepressant response to prefrontal AMPARs stimulation, we next perfused the AMPAR agonist, (S)-AMPA, bilaterally (100 µM in each hemisphere for 1 h) into the mPFC. This treatment induced a clear-cut antidepressant-like effect in the FST measured as decreased immobility and increased climbing and swimming behaviors (Fig. 6D), without altering general locomotor activity (Supplementary Fig. 1B). The effects of higher concentrations of (S)-AMPA were not examined because we had previously evidenced that they induced an overt behavioral activation with a concomitant increased locomotor activity (Amargós-Bosch et al. 2007) that would compromise the antidepressant response. In concordance with the effects of IL DBS, bilateral administration of (S)-AMPA in the mPFC significantly increased the local efflux of 5-HT (Fig. 6E) and glutamate (Fig. 6F). It is of note that bilateral perfusion of (S)-AMPA in the mPFC produced a 1.8-fold larger increase in 5-HT output than IL DBS (Supplementary Fig. 2).

Discussion

The first main finding of the present work is that the antidepressant-like effects of DBS are specific for the IL subregion of the mPFC. The same experimental condition applied more dorsally in the PL was devoid of any antidepressant response. Further, IL DBS is associated with increases of glutamate, 5-HT, noradrenaline, and dopamine in the mPFC. As extensively described (Heidbreder and Groenewegen 2003), prefrontal monoamines have been traditionally implicated in attentional processes (noradrenaline, originated in the LC), working memory (dopamine, originated in the VTA), and behavioral flexibility (5-HT, originated in the DR), conditions that are impaired in depressive states. Thus, increased serotonergic transmission in the mPFC would be associated with a better affective response whereas increased dopamine and noradrenaline release may play a role in treating cognitive and attentional deficits. In contrast to previous literature (Slatter et al. 2011), the antidepressant-like action of IL DBS cannot be attributed to a functional inactivation of the IL, or an activation of inhibitory GABAergic transmission in the IL because first, the outflow of GABA was not altered; second, a stimulated GABA transmission in the IL would likely lead to decreased glutamate efflux, which is at variance with the results.
of the present study; and third, a substantial lesion of the ventral mPFC did not produce a significant antidepressant-like effect in the FST (Hamani et al. 2010). It may seem puzzling that non-selective, local IL DBS increased glutamate transmission without increasing also GABA. Nevertheless, it has been reported that DBS could suppress activity of cell bodies and dendrites while directly stimulating nearby glutamatergic fibers (McIntyre et al. 2004; Montgomery and Gale 2008), which are predominant axonal processes in cortical structures. It is possible that differences in the prefrontal area targeted prompted the discrepancy between Slattery’s and our investigations. Thus, Slattery et al. (2011) positioned the cannulae more rostrally and laterally (probably reaching the ventral orbital cortex according to Paxinos and Watson (2005)), compared with the electrode placement in our study. On the other hand, Challis et al. (2014) reported that the optogenetic stimulation of vmPFC terminals in the DR had aversive and prodepressant effects during social defeat behavior. However, in this latter study, viral injections were carried out unilaterally in the prelimbic region of the mPFC. Therefore, the behavioral consequences of prefrontal activation (or inactivation) and the
subsequent stimulation (or inhibition) of serotonergic neurons in the DR still remains controversial and may vary if applied during versus prior to the behavioral test. Interestingly, in the present work, the antidepressant activity of DBS is associated with increased prefrontal glutamate and monoamines whereas decreases in prefrontal glutamate, noradrenaline, and dopamine did not cause more depressive behaviors. The most parsimonious explanation for the latter is that the decrease in glutamate and monoamines may be necessary, but not sufficient, for the emergence of depressive-like behaviors. Two different mechanisms (being not mutually exclusive) can account for the increase in prefrontal monoamines: first, the stimulation of the respective mPFC projections to brainstem monoaminergic nuclei, as evidenced in recent optogenetic studies (Covington et al. 2010; Warden et al. 2012; Chaudhury et al. 2013) and, second, local stimulatory effects on axons/nerve terminals. The following findings seem to support the former possibility: 1) the firing rate of DR 5-HT cells is increased after 1 h IL DBS (N. Haddjeri, personal communication); 2) cortical transection suppresses IL DBS-induced increase in prefrontal 5-HT; 3) IL DBS elevates also hippocampal 5-HT (Hamani et al. 2010); and 4) PL DBS does not increase prefrontal neurotransmitters. It is of note that prefrontal monoamine and glutamate changes follows the same time course as the behavioral effects in the FST. On the other hand, PL DBS, which results on clear-cut decreases in glutamate, noradrenaline, and dopamine, showed no antidepressant-like action. At present, we cannot provide an accurate explanation for this reduction in cortical glutamatergic and catecholaminergic outflow, but our results clearly indicate that these opposite influences of IL and PL subregions of the mPFC may likely determine the antidepressant-like activity (or its absence) after IL DBS or PL DBS, respectively. It is possible that PL cortex activates preferentially a brain region that would exert an inhibitory control over monoaminergic centers. The lateral habenula could be such region insofar it has been postulated that stimulation of the lateral habenula reduces the activity of monoaminergic nuclei of the brainstem (Sartorius and Henn 2007), which would eventually lead to decreased release in nerve endings. Although further research is needed to confirm this contention, it is worth noting that our results show a relative higher c-Fos immunolabeling of the lateral habenula after PL DBS, which could give additional support to this view.

Our c-Fos immunolabeling results also supported the view that DBS stimulates prefrontal output. However, we did not find c-Fos accumulation in the DR 2 h after IL DBS cessation. In contrast with our results, it was previously found that 1-h IL DBS induced c-Fos formation immediately thereafter (Veerakumar et al. 2014). It is possible that c-Fos induction in the DR after IL DBS has a

![Figure 5. Role of cortico-raphe projection in the effects of IL DBS. (A) One hour of IL DBS increased glutamate efflux in the DR. (B) One hour of IL DBS did not change 5-HT efflux in the DR. Repeated-measures two-way ANOVA followed by Newman–Keuls test, *P < 0.05, n = 5–6 rats per group. (C) Drawing representing the setting of the dual probe (DR + mPFC) experiment. (D) The perfusion of increasing concentrations of glutamate in the DR (expressed as percentage of baseline levels in the DR) had no effect on the local efflux of 5-HT. (E) The perfusion of increasing concentrations of glutamate in the DR elevated glutamate efflux in the mPFC. One-way ANOVA followed by Newman–Keuls test, *P < 0.05, **P < 0.01 or less, n = 7 rats per group.](http://cercor.oxfordjournals.org/Download)
very short time course. However, DBS of the IL and PL produced markedly quantitative differences in stimulated areas that might be relevant to behavioral outcomes. Thus, the excessive activation of the orbitofrontal cortex and the insula (areas known to be over-active in depression [Mayberg 1997]), together with reduction of the prefrontal glutamate release, may be responsible of the lack of antidepressant-like action after PL DBS. In contrast, comparatively much more intense c-Fos induction after IL DBS was observed in the mediodorsal and centromedial thalamic nuclei, areas strongly innervated by 5-HT projections from DR (Krout et al. 2002) that integrate affective salience and attentional resources (Metzger et al. 2010), functions that are disturbed in depression.

In the present study, we also sought to further examine the role of 5-HT in the antidepressant-like behavior of IL DBS. Our results demonstrated that the short-term antidepressant-like effects of IL DBS were not dependent on the serotonergic system because 90% depletion of 5-HT levels evoked by the 5-HT synthesis inhibitor, pCPA, did not alter the reduction of immobility and the increase of climbing in the FST caused by IL DBS. Alternatively, it is possible that 5-HT depletion higher than 90% could be needed to compromise the antidepressant response of IL DBS. This is in apparent contrast with previous findings showing that an intact serotonergic—but not noradrenergic—system is required for the antidepressant-like action of DBS (Hamani et al. 2010). In this latter setting, however, DBS had a longer duration (4 h followed 24 h later by 2 h just before FST), and was delivered 7 days after electrode implantation, which could justify the differences found. In this regard, it has recently been shown that

Figure 6. Role of AMPARs in the antidepressant effects of IL DBS. (A) Systemic (10 mg/kg i.p., 5 min before FST) and bilateral, prefrontal (300 µM, 1 h during IL DBS) administration of NBQX blocked the IL DBS-induced antidepressant-like behavior in the FST. Results are expressed as mean ± SEM of n = 6–9 rats per group (Newman–Keuls test following significant one-way ANOVA, *P < 0.05, **P < 0.001). (B) Systemic NBQX blocked the IL DBS-induced increase in mPFC 5-HT only after DBS offset. Results are expressed as mean ± SEM of n = 5–6 rats per group (Newman–Keuls test following significant two-way repeated-measures ANOVA, P < 0.05). (C) Systemic NBQX abolished completely the increase in mPFC glutamate induced by IL DBS. Results are expressed as mean ± SEM of n = 4–7 rats per group (Newman–Keuls test following significant two-way repeated-measures ANOVA, P < 0.05). (D) Bilateral intra-mPFC perfusion of 100 µM (S)-AMPA for 1 h evoked an antidepressant-like response in the FST carried out immediately thereafter. Results expressed as mean ± SEM of n = 7 rats per group (Newman–Keuls test following significant two-way repeated-measures ANOVA, *P < 0.05, **P < 0.001). (E) Bilateral intra-mPFC perfusion of 100 µM (S)-AMPA in the mPFC for 1 h increased the local concentration of 5-HT in both hemispheres. Results are expressed as mean ± SEM of n = 4–6 rats per group (Newman–Keuls test following significant two-way repeated-measures ANOVA, *P < 0.05). (F) Bilateral intra-mPFC perfusion of 100 µM (S)-AMPA in the mPFC for 1 h increased the local concentration of glutamate in both hemispheres. Results are expressed as mean ± SEM of n = 5–8 rats per group (Newman–Keuls test following significant two-way repeated-measures ANOVA, **P < 0.005).
the serotonin-induced formation of brain cytokines (which is possibly patent after 1 week of electrode implantation) may exert antidepressant actions (Warner-Schmidt et al. 2011, Perez-Caballerò et al. 2014). In addition, we also showed that cortical transection reduced the extracellular content and the IL DBS-induced increase of 5-HT in the mPFC whereas the extracellular content and the IL DBS-induced increase in glutamate were unaltered. It is thus feasible that the early antidepressant-like activity of IL DBS would rather be more dependent upon prefrontal glutamate transmission.

Given the importance of the DR and its regulation by prefrontal afferents in the pathophysiology and treatment of depression (Arango et al. 2002), we also sought to examine whether IL DBS activated this prefrontal projection in particular. Our findings showed that: first, IL DBS enhanced the efflux of glutamate in the DR without altering that of 5-HT and, second, mimicking this change in endogenous glutamate with the exogenous application of the amino acid in the DR, stimulated the efflux of glutamate and 5-HT in the mPFC. Therefore, it can be inferred that IL DBS might eventually evoke an increased excitatory prefrontal output to the DR resulting in an elevated 5-HT release in serotonergic nerve endings. As mentioned above, this is consistent with previous work showing that optogenetic stimulation of mPFC increases the output of descending projection neurons that target the DR, which is responsible for an antidepressive behavior (Covington et al. 2010; Warden et al. 2012; Chaudhury et al. 2013). It is now well established that laser stimulation of channelrhodopsin-2 over 20 Hz leads to a reduction in spike fidelity and a complete loss of firing over 40 Hz (Challis et al. 2014), which is in line with the silenced zone around the electrodes observed herein. Thus, although a role of a focal silencing of a small part of the IL in the antidepressant behavior of DBS cannot be completely ruled out, the elevated efflux of glutamate in the DR after DBS further suggests an increased prefrontal output.

The second main finding was that systemic pretreatment with the AMPAR antagonist, NBQX, abolished the antidepressant-like effects of IL DBS in the FST. Most importantly, we demonstrated for the first time that this was mimicked by prefrontal perfusion of NBQX. In parallel, NBQX completely suppressed IL DBS-induced increase in prefrontal glutamate although 5-HT was only partially blocked, notably after the offset of DBS, which possible reflects that a local stimulation of serotoninergic terminals might precede the long-range stimulation of prefrontal projection to brainstem. The concentration and dose of NBQX administered in the present study were shown previously to be devoid of any effect upon locomotor activity (Maeng et al. 2008; Lu et al. 2015). The exact concentration of NBQX in the mPFC after its systemic administration (and the subsequent level of blockade of AMPARs) was not determined. Regardless the amount of blockade of AMPARs, it is evident that DBS requires an enhanced AMPAR throughput to exert its antidepressant action. We next examined the effects of a direct activation of prefrontal AMPARs. The administration of the AMPAR agonist, (S)-AMPA (100 μM), bilaterally in the mPFC reduced the immobility and increased climbing and swimming in the FST. This was accompanied by concomitant 2-fold elevations of 5-HT and glutamate in the mPFC. Interestingly, the intra-mPFC administration of (S)-AMPA produced a larger effect on swimming behavior than IL DBS. This may be the consequence of a 2-fold increase in extracellular 5-HT (Cryan et al. 2005) and/or glutamate that would activate AMPARs (Andreasen et al. 2015). In contrast, the cortical transection also increase swimming behavior, which cannot be attributed to changes in extracellular 5-HT. In this case, the increase in swimming behavior could rather result from a substantial stimulation of AMPARs due to the considerable (−6-fold from absolute basal values) elevation of extracellular glutamate (Andreasen et al. 2015). Thus, both AMPAR activation and serotoninergic transmission might influence swimming behavior in the FST. Altogether, our results indicate that the stimulation of prefrontal AMPARs is a condition necessary and sufficient to produce an antidepressant action that might be associated to increased glutamatergic transmission in the mPFC. Therefore, we hypothesize that there is a short- and a long-term components of the antidepressant response to DBS. The long-term component would require secondary homeostatic adaptations whereas the short-term component would not and would implicate AMPAR activation. Interestingly, sustained antidepressant effects of both DBS and ketamine require an unimpaired serotoninergic system (Hamani et al. 2010; Gigliucci et al. 2013). In contrast, short-term antidepressant actions of DBS and ketamine do not require serotoninergic transmission (this work, Gigliucci et al. 2013).

In this study, we demonstrate that antidepressant-like effects of DBS are only achieved when stimulation is confined to the IL area of the mPFC. These effects are accompanied by local elevations of glutamate and monoamines. Altogether, it appears that the simultaneous prefrontal release of monoamines and glutamate play a role in the rapid beneficial effects of the stimulation. Although it has been reported that noradrenaline is not required for a more prolonged exposure of DBS, it is conceivable that the increase of prefrontal noradrenaline (and dopamine) might contribute to the beneficial effects at the onset of treatment. Our results also show that the antidepressant effects of IL DBS require the stimulation of mPFC AMPARs. A better knowledge of the mechanisms that regulate prefrontal-brainstem projections may lead to novel, less invasive, and faster-acting therapies for depression.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

Funding
This work was supported by the Junta de Andalucía (CTS-510, CTS-7748 to E.B.) and by the Instituto de Salud Carlos III, Subdirección General de Evaluación y Fomento de la Investigación (FIS grants PI10-01103 and PI13-00038 to A.A. and PI12-00915 to E.B.) that were co-funded by the European Regional Development Fund (A way to build Europe). Funding from the Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM, Intramural Project Grant P01G), and the Generalitat de Catalunya (2009SGR220) is also acknowledged. L.J.-S. was recipient of a predoctoral fellowship from the Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS).

Notes
We thank Verónica Paz for the skilful technical assistance. Conflict of Interest: None declared.

References


