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Original Article

Astroglial Control of the Antidepressant-Like Effects of Prefrontal Cortex Deep Brain Stimulation

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ABSTRACT

Although deep brain stimulation (DBS) shows promising efficacy as a therapy for intractable depression, the neurobiological bases underlying its therapeutic action remain largely unknown. The present study was aimed at characterizing the effects of infralimbic prefrontal cortex (IL-PFC) DBS on several pre-clinical markers of the antidepressant-like response and at investigating putative non-neuronal mechanism underlying DBS action. We found that DBS induced an antidepressant-like response that was prevented by IL-PFC neuronal lesion and by adenosine A_1 receptor antagonists including caffeine. Moreover, high frequency DBS induced a rapid increase of hippocampal mitosis and reversed the effects of stress on hippocampal synaptic metaplasticity. In addition, DBS increased spontaneous IL-PFC low-frequency oscillations and both raphe 5-HT firing activity and synaptogenesis. Unambiguously, a local glial lesion counteracted all these neurobiological effects of DBS. Further in vivo electrophysiological results revealed that this astrocytic modulation of DBS involved adenosine A_1 receptors and K^+ buffering system. Finally, a glial lesion within the site of stimulation failed to counteract the beneficial effects of low frequency (30 Hz) DBS. It is proposed that an unaltered neuronal–glial system constitutes a major prerequisite to optimize antidepressant DBS efficacy. It is also suggested that decreasing frequency could heighten antidepressant response of partial responders.

1. Introduction

With a lifetime prevalence of 16% in the United States, major depressive disorder (MDD) is one of the most common psychiatric disorders (Kessler et al., 2003). Despite the large range of available pharmacological treatments, they remain unsatisfactory because of their delayed onset of action (3–6 weeks) and their partial therapeutic efficacy. Indeed, 30–40% of patients are considered to have chronic and refractory forms of MDD (Berton and Nestler, 2006). Recently, high frequency deep brain stimulation (DBS) within the subcallosal cingulate gyrus (SCG) was shown to rapidly improve depressive symptoms in refractory patients, with 60% of patients being characterized as responders, including 35% of them in total remission (Mayberg et al., 2005; Kennedy et al., 2011; Lozano et al., 2012). Despite these promising and exciting results, the neurobiological bases underlying DBS therapeutic action remain largely unknown.

In rat, the anatomical connections and functional roles of the ventromedial prefrontal cortex (Gabbott et al., 2003) have led to consider this region as the equivalent of the human SCG. The stimulation of the ventromedial prefrontal cortex (vmPFC) induces antidepressant-like

Abbreviations: DBS, deep brain stimulation; fEPSP, field excitatory post-synaptic potential; HFS, high frequency stimulation; IL-PFC, infralimbic prefrontal cortex; L-AAA, L-alpha-aminoadipic acid; LFS, low frequency stimulation; PCPA, 4-chloro-DL-phenylalanine methyl ester.

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behavior in the forced swim test (FST) (Hamani et al., 2010), and reverses anhedonic-like behavior in animal models of depression (Hamani et al., 2012). Curiously, they found that the antidepressant-like behavioral response of DBS in the FST was unchanged after an ibotenic acid-induced neuronal lesion of the vmPFC, suggesting the involvement of "en-passant" fibers (Hamani et al., 2010) and/or glial cells.

Glial cells are likely implicated in the physiopathology of depression. Post-mortem studies have shown a decreased density of glial cells in the prefrontal cortex and the hippocampus of depressed patients (Cotter et al., 2001; Ongur et al., 1998). Accordingly, preclinical studies demonstrated that a loss of astrocytes in the vmPFC induces depressive-like behaviors (Banasr and Duman, 2008). More recently, it has been shown that D-serine and ATP, two astrocytic transmitters, exert antidepressant-like properties in rat and mice (Malkesman et al., 2011; Cao et al., 2013), while glial-mediated modulation of glutamate uptake in the PFC produces antidepressant-like effects by enhancing cortical glutamate availability (Banasr et al., 2010). Considering that astrocytes are implicated in the processes of neurotransmission and synaptic plasticity (Halassa and Haydon, 2010; Panatier et al., 2011), they seem to be good candidates to interfere in DBS neurobiological action. Importantly, recent in vitro data reported that DBS is associated with an increase of ATP outflow within both thalamic and cortical slices, resulting in an accumulation of adenosine, which in turn depresses excitatory transmission through A_1 receptor activation (Bekar et al., 2008).

The aims of the present study are to provide a better understanding of the neurobiological bases of DBS, and to determine the role of glial cells in the mechanism of action of DBS by using a gliotoxin specific for astrocytes, the L-alpha-amino-adipic acid (L-AAA) (Brown and Kretzschmar, 1998; Takada and Hattori, 1986). L-AAA infusion in vivo induces a transitory ablation of astrocytes (Khurgel et al., 1996; Takada and Hattori, 1986; Banasr and Duman, 2008; Lima et al., 2014) by using Na^+-dependent transporter to enter into cells and block essential cellular functions involving glutamate, such as protein synthesis and energetic metabolism (Brown and Kretzschmar, 1998). Thus, we have studied, in sham and glial-lesioned rats, the in vivo effects of infralimbic prefrontal cortex DBS (IL-DBS) on antidepressant-like behavior in the FST, serotonin (5-HT) neuronal plasticity and function, dentate gyrus mitogenesis, hippocampal synaptic metaplasticity and IL-PFC spontaneous low-frequency oscillations.

2. Materials and Methods

Materials and Methods section is detailed in Supplementary Materials.

2.1. Animals

Efforts were made to minimize the number of animals used, as well as their suffering. In this attempt, in vivo experiments were not replicated for cell proliferation and electrophysiological experiments. However, the same DBS treatment in control has been reproduced several times throughout the manuscript thereby demonstrating the reproducibility of our data. Sample size for each experiment was calculated using an alpha of 0.05 and beta of 0.20. All animals were randomized for each experiment and the experiments were blinded to the experimenter. Experiments were carried out in male Sprague–Dawley OFA rats (Charles Rivers, France) weighing from 260 to 320 g on the day of the experiment. Experiments were performed in compliance with the European Communities Council (86/609 ECC) for the care and use of laboratory animals and ARRIVE guidelines and with the approval of the Regional Animal Care Committees (University Lyon 1, CREEA Côte d'Azur).

2.2. Stimulation of the Prefrontal Cortex

To achieve IL-DBS, concentric bipolar stimulating electrodes (Rhodes Medical Instruments, USA; SNEX-100, 0.25 mm diameter) were positioned in the IL-PFC (in mm: 3.2 anterior and ±0.5 lateral to bregma; between 5.2 and 5.5 ventral from the skull) in anesthetized rats. Stimulation was conducted with an isolated pulse stimulator (model 2100, A-M Systems, USA) at 150 μA, 130 Hz and 60 μs (Hamani et al., 2010).

2.3. Cortical Infusions

The L-alpha-aminoadipic acid (L-AAA) and the ibotenic acid were infused bilaterally into the IL-PFC in anesthetized rats. All experiments were carried out 48 h after the L-AAA infusion (20 μg/μL, final dose of 100 μg/hemisphere) (Khurgel et al., 1996). The ibotenic acid (15 μg/μL) was infused 7 days before FST.

The A_1 receptor antagonist DPCPX, A_1 receptor agonist CHA and K^+Cl^- (2.7 or 30 mM) solutions were perfused within the IL-PFC through a microdialysis probe.

2.4. Immunostaining

At the end of experiments, rats were transcardially perfused to evaluate the position of the glial lesion and quantify the number of GFAP-positive cells in the IL-PFC using fluorescent labeling of GFAP and DAPI. Only animals showing lesions within the IL-PFC were included in the study. For neuronal labeling, we used NeuN immunostaining.

To evaluate cell proliferation in the dentate gyrus of dorsal and ventral hippocampi, rats were injected with BrdU (4 × 50 mg/kg i.p.) every 2 h as previously described (Mnie-Filali et al., 2011).

2.5. Behavioral Experiments

Rats received continuous unilateral stimulation during 4 h after the pre-test session of forced-swim test (FST) and 2 h before the test session, 24 after (Fig. S3). The immobility duration was assessed by image analysis through a specialized digital interface (Videotrack, ViewPoint). Rats received (i.p.) 3 injections of either caffeine (2 mg/kg), the selective antagonist of A_1 receptors DPCPX (4 mg/kg) and the selective antagonist of A_{2A} receptors SCH4424164 (1 mg/kg).

The locomotor activity was performed using actimeter (Imetronic, France).

2.6. Electrophysiological Recordings

2.6.1. 5-HT Cell Recordings

Extracellular unitary recordings of presumed DRN 5-HT neurons were performed as previously described (Mnie-Filali et al., 2011). To determine possible changes in the spontaneous firing activity of presumed 5-HT neurons induced by 1 h IL-DBS, 3–4 successive descents were performed along the DRN in each rat to find about 6 neurons before and/or 6 neurons after the stimulation.

2.6.2. Field EPSP Recordings

The amplitude of field excitatory post-synaptic potential (fEPSP) was recorded in the CA1 stratum radiatum of hippocampus as previously described (Mnie-Filali et al., 2006). LTD was induced by low frequency stimulation (LFS), which consisted on biphasic square pulses (0.2 ms duration) at 3 Hz during 5 min. After 30 min recordings, LTP was induced by high frequency stimulation (HFS) which consisted on ten trains at 0.5 Hz, each composed of 20 pulses at 200 Hz. DBS of the IL-PFC was turned-on during the LFS and HFS of the hippocampus.

These recordings were also performed in stressed rats (using elevated platform) and in 5-HT-depleted rats (using PCPA).

2.6.3. Local Field Potential Recordings

A bipolar electrode linked with a connector was chronically implanted targeting the left IL-PFC. IL-DBS was applied during 4 h the second day

after surgery and 2 h the day after. Rats were then anesthetized and oscillatory activity within the IL-PFC was recorded during 5 min.

2.6.4. Statistical Analysis

All results are expressed as mean ± S.E.M. Comparisons between groups were analyzed with analysis of variance. Statistical Analysis section is detailed in Supplementary Materials and statistical analyses for each figure are shown in Supplementary Table S1.

3. Results

3.1. Glial and Neuronal Modulations of the IL-DBS-Induced Antidepressant-Like Response in the FST

Forced-swim test is a frequently used model to screen potential antidepressants (Porsolt et al., 1977). In the present study, it was used to highlight antidepressant-like behavior induced by IL-DBS and its putative glial modulation in rats infused with vehicle or L-AAA gliotoxin in the IL-PFC. As illustrated in Fig. 1D, stimulation electrodes were implanted unilaterally in the infralimbic part of the vmPFC. Two sessions of IL-DBS (Fig. S3) significantly decreased immobility duration in rats with intact IL-PFC demonstrating an antidepressant-like effect of DBS (Fig. 1A). To determine whether IL-DBS involved the local neuronal population, its antidepressant-like effect was assessed in rats infused with ibotenic acid 7 days before the FST (Fig. 1A and B). Our results revealed that neuronal lesion had no effect by itself in the FST, as previously shown (Banasr and Duman, 2008), but robustly prevented the antidepressant-like effect of IL-DBS. Interestingly, the antidepressant-like effect of DBS was prevented by a loss of astrocytes within the stimulated area after L-AAA infusion in rat's IL-PFC (Fig. 1C). Glial lesion had no effect by itself on immobility duration and did not alter locomotor activity of rats (Fig. S4).

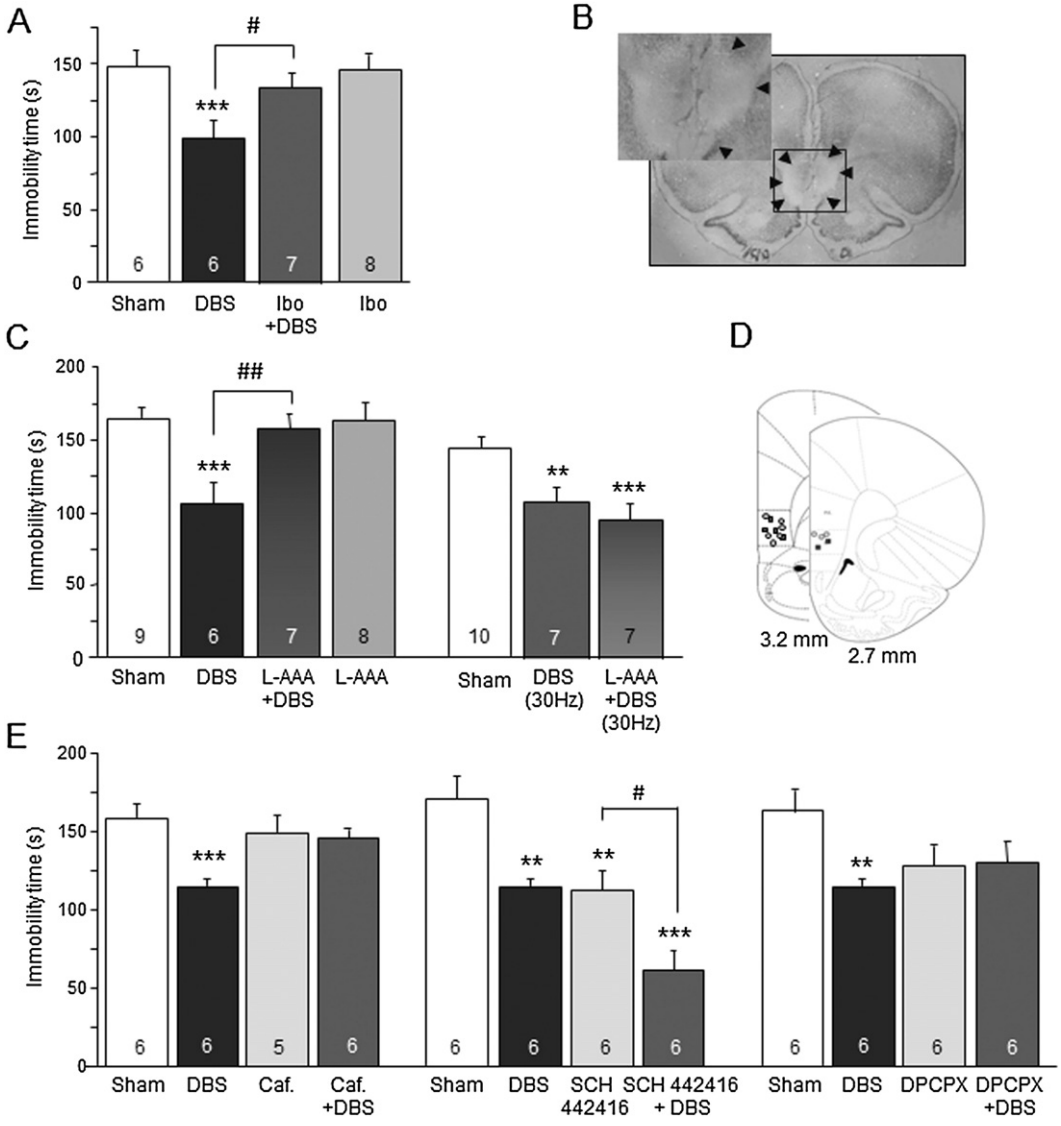

Fig. 1. Modulation of the IL-PFC DBS-induced antidepressant-like response in the FST. (A) IL-DBS induced a decrease in immobility in FST. Ibotenic acid infusion had no effect on immobility time in the FST but prevented the antidepressant-like effect of IL-DBS. (B) Representative photomicrograph of a coronal NeuN-immunostained section containing the IL-PFC from ibotenic-treated rats. Black arrows represent the outline of the area where a complete disappearance of NeuN-immunoreactivity was observed after an ibotenic acid infusion in IL-PFC. (C) IL-PFC glial lesion counteracted the decrease in immobility in FST induced by high frequency IL-DBS (130 Hz) but did not prevented the antidepressant-like effect induced by low frequency IL-DBS (30 Hz) (**p < 0.01 and ***p < 0.001 vs sham, ##p < 0.01 vs IL-DBS). (D) Schematic representation of coronal brain sections showing the location of implanted electrodes in DBS- (130 Hz) (black squares) or L-AAA + DBS- (130 Hz) (gray dots) treated animals for the Fig. 1C (left) groups. (E) Caffeine (Caf.) abolished the decrease of immobility induced by IL-DBS in FST. SCH442416 induced a reduction of immobility on its own in the FST, but failed to prevent the IL-DBS-induced antidepressant-like effect. DPCPX abolished the decrease of immobility induced by IL-DBS in FST (**p < 0.01 and ***p < 0.001 vs vehicle and #p < 0.05). Results are expressed as mean ± SEM of immobility duration. Numbers at the bottom of the columns represent the number of rats tested per group.

To further support the local L-AAA lesion effect, we replicated these experiments after a functional disruption of the astrocytic syncytium using the non-specific blocker of astroglial gap junction carbenoxolone (Juszczak and Swiergiel, 2009; Sun et al., 2012). Importantly, we found that gap junction blockade at the site of stimulation also prevented the antidepressant effect of IL-DBS (Fig. S5). Similarly, to further probe a role of glia cells on the effects of IL-DBS in the FST, we replicated these experiments under lower frequency IL-DBS, i.e., 30 Hz. Our results showed that IL-DBS at 30 Hz significantly decreased immobility duration in sham rats revealing an antidepressant-like effect. Importantly, the latter effect of IL-DBS at 30 Hz was not modified by a glial lesion (Fig. 1C). Noteworthy, neither glial lesion nor electrode implantation affected by themselves time immobility in the FST, such results contrasted with previous studies (Banasr and Duman, 2008; Lee et al., 2013; Perez-Caballero et al., 2013) mainly because of the use of a different technical procedure (see Figs. S6 and S8).

3.2. Effects of Adenosine A_1/A_{2A} Receptor Antagonists on IL-DBS Antidepressant-Like Response in the FST

Kaster et al. (2004, 2012) have previously shown that adenosine A_1 agonists (injected i.c.v. and i.p.) induce antidepressant-like effects in the FST. Considering that DBS is associated to the accumulation of adenosine (Bekar et al., 2008), we investigated the involvement of adenosine transmission in DBS-induced behavioral effects. Systemic injections of caffeine, which is a non-selective antagonist of adenosine receptors, prevented the decrease of immobility in the FST induced by IL-DBS (Fig. 1E) without affecting spontaneous locomotor activity of rats (Fig. S4). To further characterize this effect, the A_{2A} receptor antagonist SCH442416 and the A_1 receptor antagonist DPCPX were then administered in control and stimulated rats (Fig. S3). Although the immobility time was significantly decreased in SCH442416-injected rats compared to sham, the antidepressant-like effect of IL-DBS was still significantly present in rat pre-treated with SCH442416 (Fig. 1E). Given that SCH442416 significantly decreased locomotion (Fig. S4), SCH442416 induced an antidepressant-like response that was not attributable to an increase in locomotor activity. On the other hand, the administration of the A_1 receptor antagonist DPCPX significantly prevented the antidepressant-like effect of DBS without altering spontaneous locomotion (Fig. S4).

3.3. Glial Modulation of IL-DBS-Induced Enhancement of Hippocampal Mitogenesis

The neurogenic hypothesis of depression postulates that decreased production of new granule cells in the DG is linked to the pathophysiology of depression and that increased hippocampal neurogenesis is required for the behavioral effects of antidepressant treatment (Malberg et al., 2000; Santarelli et al., 2003). In the present study, the effect of 2 h IL-DBS (130 Hz, 150 μA bilaterally) on dorsal and ventral hippocampal mitogenesis was evaluated after BrdU labeling (Fig. 2A, Fig. S3). Remarkably, IL-DBS significantly increased mitogenesis in the dentate gyrus of dorsal and ventral hippocampi, an effect that was significantly prevented by a glial lesion within the stimulated area (Fig. 2B). Noteworthy, the glial lesion had no effect by itself on mitogenesis in the hippocampus, a result that correlated well with the lack of depression-like effect of the L-AAA infusion.

3.4. Glial Modulation of IL-DBS-Induced Changes on Synaptic Metaplasticity in Dorsal Hippocampus

Hippocampal synaptic plasticity is considered as a reliable preclinical parameter allowing the characterization of antidepressant-induced changes in the molecular and physiological events that underpin the

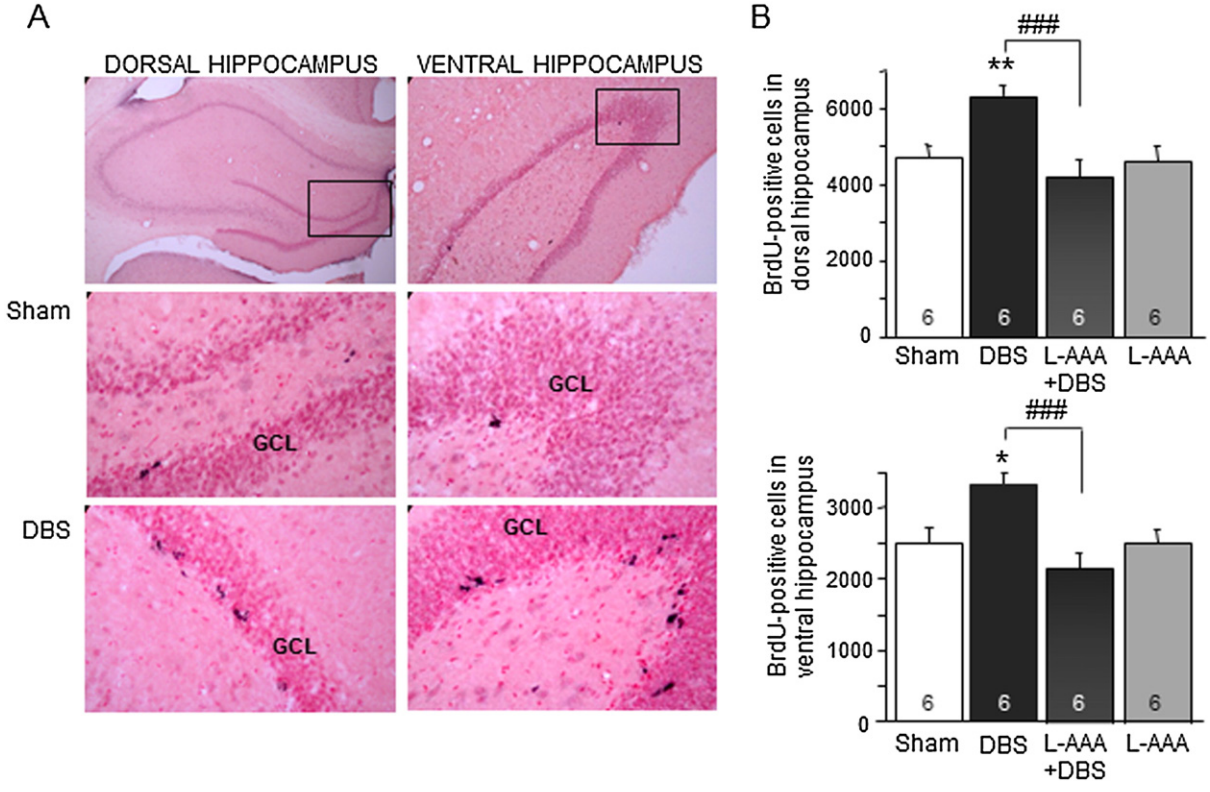

Fig. 2. Effects of a local glial lesion on IL-DBS-induced enhancement of dentate gyrus mitogenesis. (A) Representative photomicrographs of dorsal and ventral hippocampi (magnification: in top ×10 and bottom ×40, enlarged view of boxed area in top) of sham and DBS-treated rats. BrdU-positive cells (black) were seen in granule cell layer (GCL). (B) IL-DBS increased mitogenesis in dorsal and ventral hippocampi (*p < 0.05, **p < 0.01 vs sham), an effect counteracted by L-AAA infusion (#p < 0.05, ###p < 0.001 vs DBS-treated rats). Numbers at the bottom of the columns represent the number of rats per group.

regulation of synaptic connectivity related to their therapeutic efficacy (Berton and Nestler, 2006; Pittenger and Duman, 2008). Hence, the modulation of hippocampus synaptic metaplasticity by IL-DBS and glial lesion was examined in vivo by application of low and high frequency stimulations (LFS and HFS) on Schaffer's collateral pathway to induce long-term depression (LTD) or long-term potentiation (LTP), respectively, in hippocampal CA1 area (Fig. 3A inset).

As expected in dorsal hippocampus of naïve rats, LFS failed to induce a LTD and HFS resulted in a LTP of approximately 30% (Fig. 3A and C). Interestingly, while concomitant IL-DBS did not modify field excitatory post-synaptic potential (fEPSP) after LFS, it blocked the LTP induction after HFS, a result that is consistent with previous data showing a decrease of LTP after acute antidepressant administrations (Shakesby et al., 2002; Mnie-Filali et al., 2006). Notably, L-AAA infusion within the IL-PFC significantly reduced the LTP after HFS compared to sham and prevented IL-DBS-induced blockade of LTP after HFS (Fig. 3A and C). Statistical analysis detected a significant interaction between the effects of IL-DBS and L-AAA infusion demonstrating that the effect of IL-DBS on synaptic plasticity is dependent of an intact glial system.

To further characterize the modulation of hippocampus synaptic metaplasticity by IL-DBS and glial lesion, we replicated these experiments in stressed rats (Fig. 3B). An acute stress was induced by placing animals on a circular elevated platform for 30 min before electrophysiological recordings. As expected in the dorsal hippocampus of stressed rats (Xu et al., 1997), LFS induced a stable LTD of approximately 17% whereas HFS failed to elicit a LTP (Fig. 3B and C). Interestingly, concomitant IL-DBS reversed the effects of stress on both LTD and LTP. Importantly in L-AAA-infused rats, IL-DBS failed to counteract the LTD and LTP-blockade induced by stress (Fig. 3B and C).

To characterize the involvement of the 5-HT system in the modulation of hippocampal metaplasticity by IL-DBS, a serotoninergic depletion was performed using the 5-HT synthesis inhibitor PCPA. PCPA pre-treatment induced LTD after LFS and prevented LTP induction after HFS on its own. Importantly, DBS failed to further decrease AUC in PCPA-treated rats suggesting that serotoninergic depletion totally prevented the decrease of LTP induced by IL-DBS (Fig. 3D). Statistical analysis revealed a significant interaction between the effects of IL-DBS and serotonin depletion after HFS, suggesting that IL-DBS-induced effects on synaptic plasticity are modulated by serotonin.

3.5. Glial Modulation of IL-DBS-Induced Enhancement of Slow Oscillatory Activity Recorded in the IL-PFC

Disrupted low oscillatory activity has been reported in animal model of depression (Voget et al., 2015; Gazit et al., 2015). As illustrated in Fig. 4, analysis of spontaneous local field potentials and relative power spectra revealed that IL-DBS increased low frequency bands (0–21 Hz) within the IL PFC compared to sham rats (Fig. 4A and B). Analysis of the percentage of total power spectrum (Fig. 4C) showed that IL-DBS significantly enhanced the δ, θ, α, and β frequencies in comparison to non-stimulated animals. Importantly, the latter effect was significantly abolished in L-AAA infused animals while a glial lesion had no effect by itself on slow waves activities recorded in the IL-PFC.

3.6. Glial Modulation of IL-DBS-Induced Enhancement of the Spontaneous Activity of Presumed DRN 5-HT Neurons

Classical antidepressants are well known to enhance serotoninergic neurotransmission, with a time course that is consistent with the onset of their therapeutic effects (Blier and de Montigny, 1994). In the present study, the firing activity of presumed 5-HT neurons was recorded in the dorsal raphe nucleus (DRN) before and/or after 1 h of stimulation of the IL-PFC (30 and 130 Hz, 150 μA bilaterally) in anesthetized rats (Fig. S3).

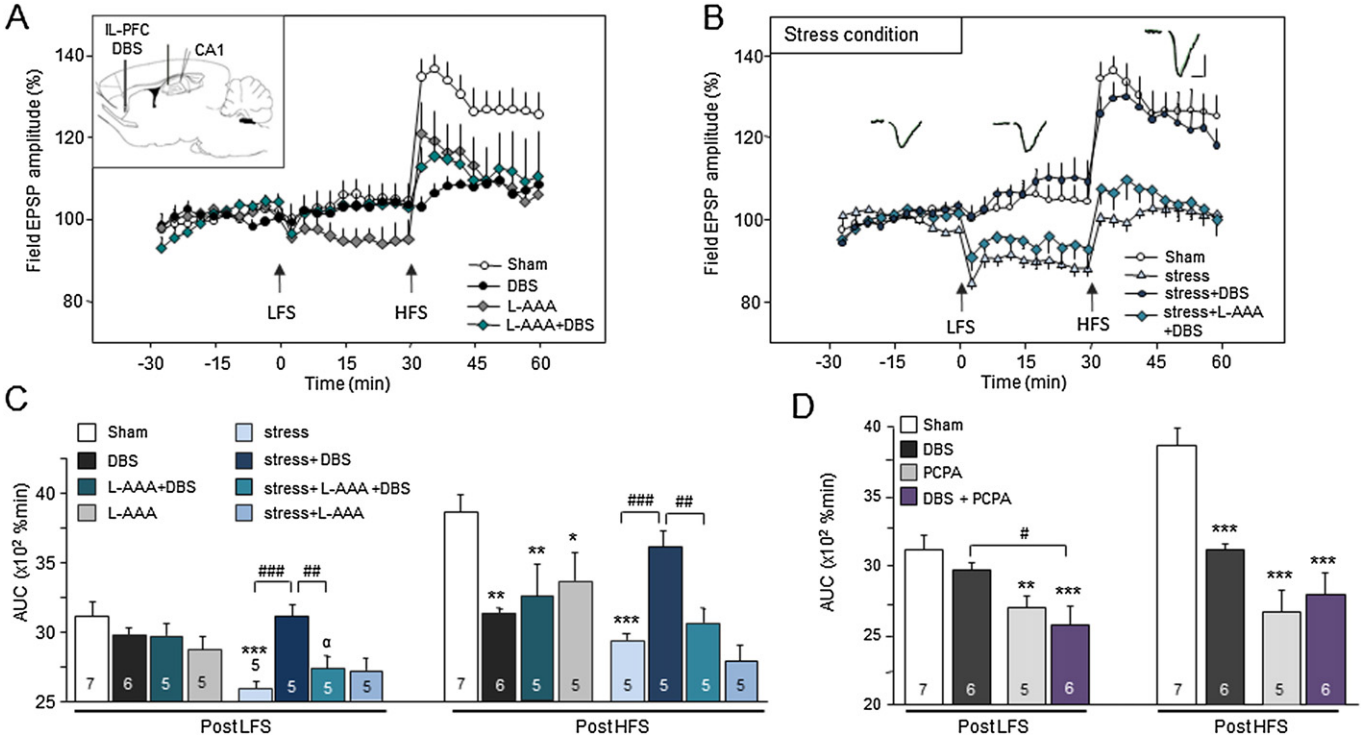

Fig. 3. Effects of a glial lesion on IL-DBS-induced changes in hippocampal synaptic metaplasticity. (A) Inset: schematic representation of the experimental protocol showing a stimulating and a recording electrode in the hippocampus and a DBS electrode in the IL-PFC. Time-course responses illustrate changes induced by DBS and glial lesions in naïve rats. (B) Time-course responses in stressed rats illustrating the effects of glial lesion on DBS modulation of synaptic plasticity. Insets show typical field EPSPs recorded before and after low and high frequency stimulations; calibration vertical bar, 0.5 mV; horizontal bar, 5 ms. (C) Area Under the Curve (AUC) histograms illustrate changes induced after LFS or HFS by IL-DBS and/or glial lesion in sham and stressed rats. (D) AUC histograms illustrate changes induced after LFS or HFS by IL-DBS and/or PCPA injections in naive rats. Numbers at the bottom of the columns represent the number of rats per group ($^{*}p < 0.05$ $^{**}p < 0.01$ and $^{***}p < 0.001$ vs naïve sham. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.01$ vs DBS-treated group, $^{\alpha}p < 0.05$ vs stress group).

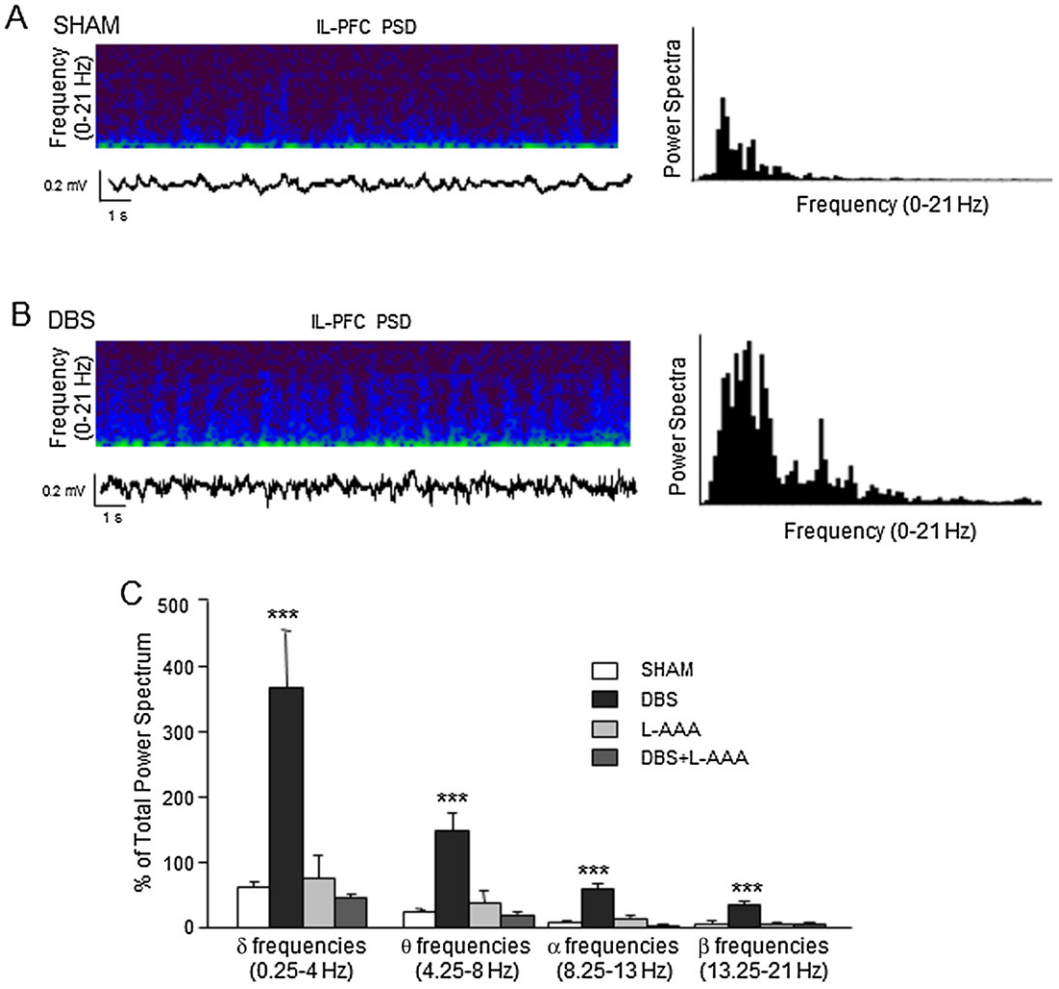

Fig. 4. Effects of a glial lesion on IL-DBS-induced enhancement of slow oscillatory activity. Spontaneous local field potentials and relative power spectra of low frequency bands (0–21 Hz) within the IL PFC in sham (A) and IL-DBS-treated rats (B). (C) Percentage of total power spectrum showed that IL-DBS significantly enhanced the δ, θ, α, and β frequencies, an effect abolished by glial lesion (***$p < 0.001$ vs sham, $n = 4$–5 rats per group).

In contrast with the data from Srejic et al. (2015) using only 5 min of DBS, 1 h of IL-DBS significantly increased the spontaneous activity of presumed 5-HT neurons by 34% in physiological conditions ($[K^+] = 2.7$ mM, Fig. 5A and B). This effect was current-dependent since 1 h stimulation at 20 μA and 130 Hz had no effect on presumed 5-HT neuronal spontaneous firing [1.1 ± 0.02 Hz before DBS ($n = 37$) vs 1.2 ± 0.02 Hz post-DBS ($n = 32$), $p > 0.05$ Student's *t*-test].

In L-AAA-treated rats the firing activity of presumed 5-HT neurons was surprisingly enhanced (Fig. 5B). However, analysis of firing patterns revealed that the latter firing rate increase was associated with the enhancement of burst firing activity of 5-HT neurons [percent of spike in burst in sham rats: 14.2 ± 2.6%, $n = 100$; after 1 h DBS: 11 ± 2.9%, $n = 71$; in L-AAA rats: 27.7 ± 0.59%, $n = 59$; after 1 h DBS: 32.6 ± 0.81%, $n = 48$; $p < 0.001$, Table S1]. In the same way, IL-PFC inactivation with carbenoxolone identically induced an increase of 5-HT firing rate [0.97 ± 0.06 Hz in sham rats ($n = 100$); 1.35 ± 0.13 Hz in cbx-treated rats ($n = 41$), $p = 0.02$ Student's *t*-test] and an aberrant increase of phasic activity of 5-HT neurons [percent of spike in burst in sham rats: 14.2 ± 2.6%; in cbx rats: 32.4 ± 11.2%; $p = 0.027$, Table S1] without altering emotional behavior in the FST (Fig. S4).

To mimic the enhancement of extracellular K^+ concentration induced by glial lesion, we perfused high concentration of KCl in the aCSF with a microdialysis probe implanted in the IL-PFC and we assessed the effect of IL-DBS on 5-HT neuronal firing activity (infusion of KCl performed during DBS and recordings). Similarly to L-AAA glial lesion, in presence of elevated $[K^+]$ (30 mM), the spontaneous firing rate of presumed 5-HT neurons was significantly enhanced and, remarkably, the facilitating effect of high frequency IL-DBS on 5-HT firing activity was also prevented (Fig. 5B). Importantly, the enhancing effect of DBS-30 Hz on 5-HT neuronal firing was not altered by the glial lesion [1.57 ± 0.10 Hz in L-AAA-treated rats ($n = 42$); 2.03 ± 0.14 Hz in IL-DBS + L-AAA-treated rats ($n = 39$), $p = 0.01$].

Since we demonstrated that adenosine A_1 receptors were involved in the antidepressant effect of IL-DBS in the FST, we have explored the effects of the selective A_1 receptor antagonist DPCPX infused via a microdialysis probe within the IL-PFC. High concentration of DPCPX (100 μM) infused during recordings (without IL-DBS) increased by itself 5-HT firing rate in sham animals [0.97 ± 0.12 Hz before DPCPX ($n = 37$) vs 1.4 ± 0.13 Hz post-DPCPX ($n = 33$), $p < 0.05$ Student's *t*-test]. As demonstrated above, 1 h of high frequency IL-DBS significantly increased by 40% the firing activity of presumed 5-HT neurons, as well as 1 h of low frequency IL-DBS (30 Hz, Fig. 5C). Remarkably, high concentration of DPCPX (100 μM), infused during the stimulation, significantly prevented the high frequency (130 Hz) DBS-induced enhancement of firing activity of presumed-5-HT neurons (Fig. 5C) but failed to prevent the enhancing effect of lower frequency IL-DBS (30 Hz) on the spontaneous activity of presumed 5-HT neurons (Fig. 5C). In the same way, the non-selective adenosine receptor antagonist caffeine (2 mg/kg, i.p.) increased by itself 5-HT firing rate [0.98 ± 0.13 Hz before caffeine ($n = 37$) vs 1.90 ± 0.19 Hz post-caffeine ($n = 27$), $p < 0.001$]. Nevertheless, there was no additive effect between IL-DBS-induced firing enhancement and caffeine-induced increased 5-HT

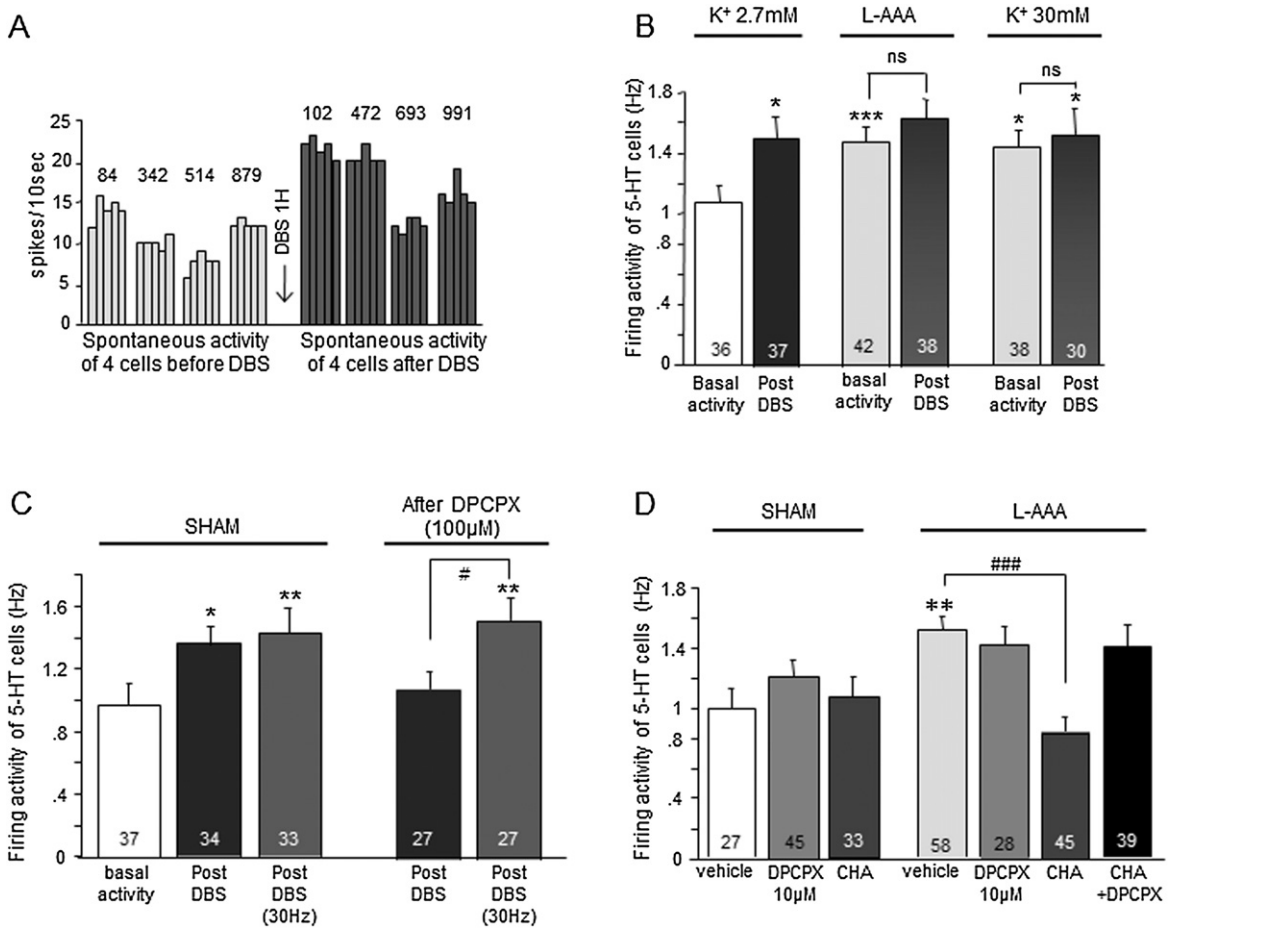

Fig. 5. Effects of IL-DBS on 5-HT neuronal firing. (A) Integrated firing rate histograms of 5-HT neurons recorded in one descent before and one descent after IL-DBS. Number above histograms: µm depth of the recorded neurons from sylvius aqueduct. (B) High frequency IL-DBS enhanced 5-HT neuronal firing in condition of normal K^+ concentration (2.7 mM). Glial lesion performed with L-AAA or K^+ high concentration (30 mM) infusion increased 5-HT neuronal activity and counteracted the effect of IL-DBS. *$p < 0.05$ and ***$p < 0.001$ vs basal activity of K^+ 2.7 mM infused group; ns = non-significant (C) IL-DBS (130 and 30 Hz) increased 5-HT neuronal firing, an effect prevented by DPCPX for high frequency IL-DBS. *$p < 0.05$ and **$p < 0.01$ vs basal activity; #$p < 0.05$ (D) Infusion of CHA reversed the effect of the glial lesion (###$p < 0.001$ CHA vs vehicle in L-AAA-treated rats), an effect counteracted by the A_1 receptor antagonist DPCPX. Numbers in the columns represent the number of 5-HT cells recorded per group (*$p < 0.05$ vs sham).

firing [1.35 ± 0.11 Hz after IL-DBS (n = 34) vs 1.43 ± 0.15 Hz post-IL-DBS + caffeine (n = 27), $p > 0.05$].

We then hypothesized that the enhancement of the firing rate of 5-HT neurons observed in L-AAA infused rats was due to the absence of an inhibitory tone on glutamatergic neurons of IL-PFC exerted by astrocytes through adenosine A_1 receptors. To test this hypothesis, the adenosine A_1 receptor agonist CHA (10 µM) and antagonist DPCPX (10 µM) were locally administered in IL-PFC via a microdialysis probe during DRN 5-HT neurons recording. In control rats, neither CHA nor DPCPX (at low dose of 10 µM) altered 5-HT firing rate in sham animals (Fig. 4D, left). Interestingly in L-AAA-treated rats (Fig. 4D, right), CHA decreased the L-AAA-induced enhancement of firing rate of 5-HT neurons. Moreover, co-administration of DPCPX prevented this effect. These results indicate that IL-PFC A_1 receptors exhibited increased sensitivity to CHA in L-AAA infused rats. Note that if the adenosine A_1 agonist CHA (10 µM) did not alter by itself 5-HT firing rate in sham animals (Fig. 4D left), CHA enhanced the action of IL-DBS at 130 Hz when it was infused during the stimulation [1.358 ± 0.11 Hz post-DBS (n = 34) vs 1.92 ± 0.15 Hz post-DBS + CHA (n = 27), $p = 0.003$ Student's *t*-test]. The obtained results clearly indicate for the first time that IL-PFC A_1 receptor is an important and labile modulator of the DBS action in controlling DR 5-HT neurons.

We then sought to assess the molecular bases underlying the DBS-induced enhancement of 5-HT neurons firing activity, by addressing the possibility that rapid synaptic growth and/or modifications may account for the observed effects. Indeed, in our experimental conditions, the increased 5-HT firing rate was observed up to 1 h after the end of DBS. It is very unlikely that such a long-lasting action could be related to increased levels of an excitatory transmitter (i.e., glutamate) at the vicinity of 5-HT cell bodies because synaptic washout occurs within seconds. However, a synaptic reinforcement at the level of the dorsal raphe might be involved in the excitatory effect described above. To test this hypothesis, PSD-95 and synapsin mRNA expression were quantified by using quantitative RT-PCR on micro-dissected samples of raphe. These two proteins are known as markers of neuronal post- and pre-synaptic plasticity, respectively (Sheng and Kim, 2002). One hour of DBS was associated with enhanced relative expression levels of both PSD-95 and synapsin mRNA in the DRN (Fig. S7). Interestingly, these effects were suppressed after IL-PFC infusion of L-AAA. This increase in mRNA level is associated with an enhanced protein level expression only for PSD-95. A non-significant decrease in synapsin protein level expression was observed. Together, these results suggested that post-synaptic neuronal markers were up-regulated within the DRN in response to acute DBS.

3.7. L-AAA Infusion Reduced the Number of GFAP-Positive Cells

In agreement with recent data (Lima et al., 2014), our study reports that L-AAA infused within the IL-PFC induced, 48 h later, a robust loss of GFAP-positive cells close to the infusion area (Fig. S1A and B). Comparison between vehicle- and L-AAA-infused rats showed a significant decrease of 70% of the number of GFAP-positive cells/mm^2 confined to

the rostro-caudal limits of the IL-PFC (3.7 mm to 3 mm from bregma; Fig. S1B) since astrocyte numbers were not affected in front of and behind the gliotoxin infusion area. Notably, NeuN immunostaining within IL-PFC clearly demonstrated no difference in the number of NeuN-positive cells in L-AAA-infused rats compared to sham (Fig. S2), indicating that L-AAA did not induce neuronal loss within the IL-PFC 48 h after local infusion.

4. Discussion

Astrocytes seem to be good candidates to interfere in the DBS neurobiological action (Vedam-Mai et al., 2012) since they are implicated in the processes of neurotransmission and synaptic plasticity (Panatier et al., 2011; Halassa and Haydon, 2010). Accordingly, Bekar et al. (2008) have shown in vitro that DBS is associated with an increase of ATP outflow within the thalamus, resulting in an accumulation of adenosine, which, in turn, depresses excitatory transmission through A_1 receptor activation. Astrocytes are indeed able to release gliotransmitters, among which ATP which is mainly considered as an excitatory transmitter. However, ATP is also rapidly hydrolyzed into adenosine, which has been reported to act as a powerful inhibitor of excitatory transmission/neuronal communication through the stimulation of adenosine A_1 receptors (Pascual et al., 2005). Interestingly, our in vivo electrophysiological results demonstrate that the administration of the A_1 receptor antagonist DPCPX or caffeine enhanced by itself 5-HT neuronal firing. It is likely that such an A_1-dependent control of 5-HT neurons is mediated, at least in part, by a modulation of the glutamatergic descending projections from the IL-PFC. Indeed, both electric (Celada et al., 2001) and optogenetic (Warden et al., 2012) stimulations of these projections have revealed that they primarily exert a stimulatory effect on 5-HT cell bodies. In addition, a very recent study using both electrophysiological and optogenetical techniques, demonstrates a "hyperdirect" excitatory PFC pathway providing a direct excitatory control of 5-HT neurons, most likely important for proper activity of the 5-HT system (Pollak Dorocic et al., 2014). Our results suggest therefore indirectly the existence of an A_1 mediated inhibitory tone on PFC pyramidal cells. Moreover, both a glial loss (with L-AAA) and a glial inactivation (with cbx) in the IL-PFC also induced an increase of presumed 5-HT neuronal activity, an effect that appears to be related to an "aberrant" enhancement of the bursting activity of 5-HT neurons. It is possible that such a paradoxical increase is, at least in part, consequent to a disinhibition, related to the suppression of this A_1-dependent tonus as revealed by the presence of the sensitization of A_1 receptors.

To date, the recently proposed hypothesis deciphering the mode of action of DBS points toward a functional inactivation of neuronal activity in the stimulated area, and an excitation of direct axonal and fiber pathways near the electrodes that would regulate distant structures (Montgomery and Gale, 2008; Vitek, 2002). In support to this hypothesis, our study demonstrated that high frequency stimulation of the IL-PFC produced a current-dependent increase of the presumed DRN 5-HT neuronal firing rate, suggesting an increased glutamatergic output from IL-PFC projections (Peyron et al., 1998; Celada et al., 2001). In agreement with a recent study (Veerakumar et al., 2014), our results showed that acute IL-DBS increased synaptogenesis within the DRN, an effect that should favor the glutamatergic tone exerted by IL-PFC terminals on serotoninergic neurons. Indeed, increased mRNA for the postsynaptic marker PSD-95 and the presynaptic protein synapsin were observed in the DRN of stimulated rats. In the same way, increased PSD-95 protein level indicate that this marker of postsynaptic density is increased within the DRN, suggesting that processes of synaptic growth have been triggered by vmPFC DBS. Besides, the fact that mRNA and protein levels of synapsin appear to be modulated in an opposite way by the stimulation suggest that the synthesis of this factor has increased within DRN cells, but that the protein itself has started to migrate outside the observed brain structure. This is in agreement with the presynaptic nature of synapsin as a promoter of vesicular formation, and would indicate that 5-HT neurons are more activated and more mobilized to release their transmitter after DBS. This explanation is fully compatible with the fact that both behavioral (i.e., in the forced swim test) and electrophysiological (i.e., on 5-HT neurons) effects of IL-DBS were still present after the stimulator had been turned off. This enhancement of 5-HT neurotransmission might be implicated in DBS-induced effects on dorsal and ventral hippocampi. Indeed, we demonstrated that the capacity of IL-DBS to block LTP induction depends on the integrity of the 5-HT system since 5-HT depletion produced by PCPA abolished this effect. Accordingly, it was shown that 5-HT is able to potentiate synaptic transmission through the activation 5-HT_4 receptors (Kobayashi et al., 2008) which are known to play a key role in the regulation of synaptic plasticity in CA1 (Kemp and Manahan-Vaughan, 2004, 2005) and to influence learning and memory (Lamirault and Simon, 2001; Moser et al., 2002). In the same way, it has been reported that the effects of DBS in the FST are completely abolished in 5-HT lesioned rats (Hamani et al., 2012). Hence, these results strongly suggested that the effects of IL-DBS on synaptic plasticity and mitogenesis are mostly mediated by 5-HT and that the integrity of 5-HT system is crucial for the antidepressant efficacy of DBS.

The present study reveals that an ibotenic acid lesion within the IL-PFC prevented the antidepressant-like effect of DBS, showing that local neuronal population is implicated in the effects of the stimulation, and is not just "inactivated" as currently hypothesized. When activated by DBS, pyramidal neurons and their axons would release an augmented quantity of glutamate, leading to an over-activity of presumed 5-HT neurons within the DRN and an increase of 5-HT release in terminal areas (Fig. 6). Taken together, these results support the view that the antidepressant-like effect of a cortical stimulation might be mediated by both the activation of local neuronal population and the excitation of axons and passing fibers that would enhance, among others, 5-HT neurotransmission (A more detailed discussion on this "excitation vs inhibition theory" is provided in the Supplementary Material). Accordingly, we demonstrated that the antidepressant-like effect of IL-DBS was associated with an increase of slow oscillatory activities within the stimulated area, an effect prevented by local L-AAA infusion. A similar increase has been observed following ECT therapy, associated with an augmentation of IL-PFC synaptogenesis and gliogenesis (Bouckaert et al., 2014). Together, these findings raise the interesting hypothesis that fast-acting AD strategies share the common feature of re-enforcing intra-IL-PFC connections, which in turn may strengthen the positive modulation exerted by pyramidal neurons on their subcortical targets like the DRN. If so, the involvement of astrocytes in the AD effects of DBS, would not be surprising given their importance in the processes of synaptic plasticity and reinforcement.

Another most prominent and studied role of astrocytes is their ability to maintain the potassium homeostasis, by actively pumping K^+ ions from the extracellular level, thus preventing them to accumulate due to neuronal activity (Kofuji and Newman, 2004; Noori, 2011; Larsen and MacAulay, 2014). Accordingly, the glial K(ir)4.1 channel is crucial for the maintenance of the resting membrane potential of glial cells and play a main role in the homeostasis of extracellular potassium as previously also shown in vivo in glial-conditional knock-out K(ir)4.1 mice (Chever et al., 2010). For this reason, we also hypothesized that an increased extracellular concentration of K^+ mimicked the effect of a glial lesion. Hence, we used reverse microdialysis to perfuse the IL-PFC with a K^+-enriched aCSF (Fig. 5B). The obtained results confirmed our working hypothesis, as 5-HT neuronal firing rate in high intra-mPFC [K^+] condition was very similar to that of L-AAA lesioned rats, both in basal situation and after DBS. One may assume that an alteration of astrocyte function within the lesioned site would lead to an increase of extracellular [K^+], which in turn would produce a depolarization of IL-PFC neuron membrane. In the absence of any stimulation, these effects are expected to facilitate pyramidal neuron activity within the mPFC, and consequently in its projection areas such as the dorsal raphe. This is precisely what is observed in non-stimulated animals. However, the

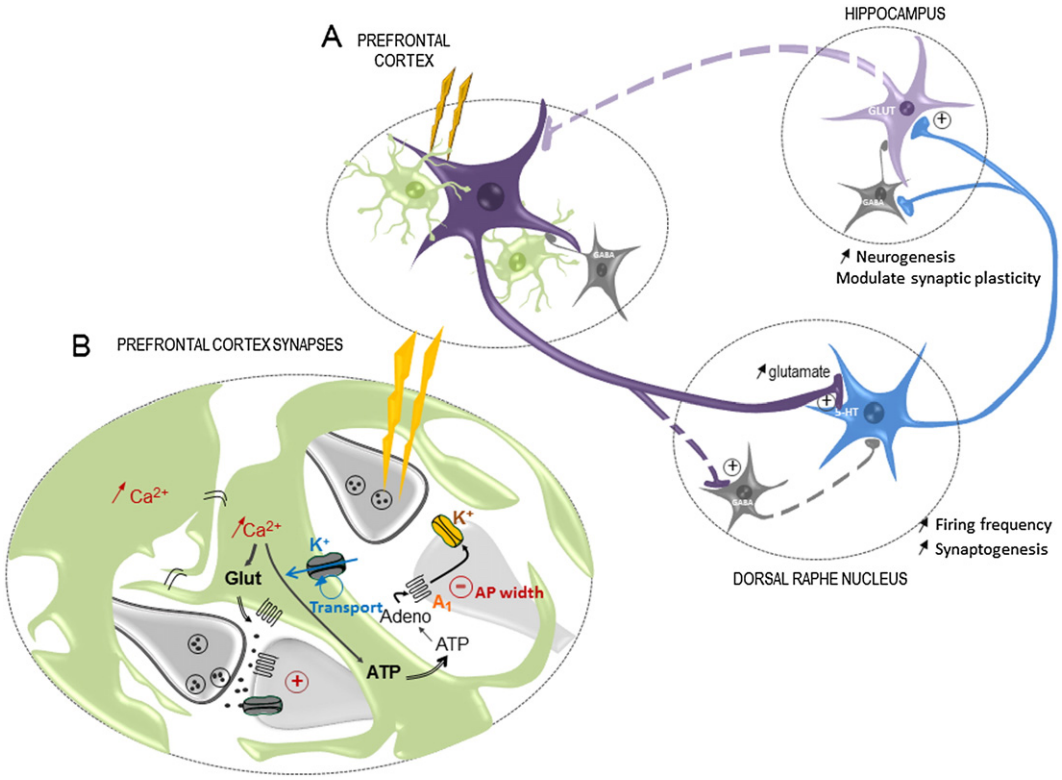

Fig. 6. A. Schematic representation of our working hypothesis on the mechanisms of action of IL-DBS. IL-DBS would activate glutamatergic neurons and increase glutamate concentration within the dorsal raphe. This would enhance the firing rate 5-HT neurons. Consequently, 5-HT release is enhanced in the hippocampus, influencing neurogenesis and synaptic plasticity. B. Astrocytes, by releasing gliotransmitters (such as glutamate and ATP), communicate with neurons at the synapse. Glutamate stimulates neuronal synaptic release and would contribute to the activation of post-synaptic receptors. ATP is rapidly hydrolyzed into adenosine (adeno), which would increase the stimulation of adenosine A_1 receptors and, in turn, should result on a K^+ channel-mediated reduction of the late hyperpolarization phase of action potentials (Sasaki et al., 2011). Ultimately, the resulting temporal shrinking of action potentials (AP width, *in orange*) may help the neuron to sustain the high frequency demand related to IL-DBS. Astrocytes also maintain the potassium homeostasis, by actively pumping K^+ ions from the extracellular level thus preventing them to accumulate due to neuronal activity (*in blue*). A loss of astrocytes should therefore lead to an increase of extracellular [K^+], which in turn would produce a depolarization of IL-PFC neuron membrane. This would facilitate basal pyramidal neuron activity, but, due to a "ceiling" phenomenon, would also likely impair the ability of pyramidal cells to respond to the phasic, high-frequency solicitation related to DBS. Actually, an increase of extracellular [K^+] mimicked a glial lesion effect.

depolarizing action of an elevated extracellular [K^+] is also likely to impair the ability of pyramidal cells to respond to the phasic, high frequency solicitation demanded to sustain the stimulation at 130 Hz. Again, the obtained results, showing that 130 Hz DBS is unable to further affect 5-HT activity in the presence of 30 mM of K^+, are in support of this latter hypothesis.

Also, it appears paradoxical to note that adenosine, via A_1 receptors, can inhibit cell firing under basal condition (as revealed by the disinhibition with DPCPX and caffeine) and can also participate in sustaining firing under high frequency of stimulation (as revealed with the potentiating action of CHA). Interestingly, a study aiming at dissecting the role played by A_1 receptors in the shape of action potentials and in the regulation of axonal conductance reveals in vitro that the administration of DPCPX increases the width of axonal action potentials, suggesting that astrocytes, through the release of adenosine and subsequent A_1 receptor stimulation, might be able to modulate the shape of axonal action potentials (Sasaki et al., 2011). The authors show that this latter effect is due to the modulation of the voltage-activated K^+ channels responsible for neuronal after hyperpolarization. As suggested, a temporal shrinking of action potentials can be beneficial when the neuron is solicited in response to high-frequency stimulations, permitting to sustain a bursting activity that requires very short inter-spike intervals. However, when the neuron fires at a low frequency, the increased late-phase hyperpolarizing K^+-current induced by A_1 stimulation (Sasaki et al., 2011) should have only marginal inhibitory effects, which would account for the results in basal conditions. In accordance with these latter hypotheses and results, we demonstrated that the effects of astroglial lesion on IL-DBS-induced effects are frequency-dependent. Under anesthesia, the resting frequency of mPFC pyramidal neurons is around 2 Hz (Llado-Pelfort et al., 2012), and imposing a frequency of 30 Hz already constitutes a potent paradigm to solicit their excitation. In the absence of astrocytes, the depolarization of neuron membrane related to K^+ accumulation did not reach a supra-threshold, "depolarization block-like" level, and that pyramidal neurons were still able to follow a moderate DBS at 30 Hz contrary to DBS at 130 Hz. Taken together, putative mechanisms involved in the influence of a glial lesion on IL-PFC and DRN 5-HT functions can be suggested. They are both based on the multiple controls that K^+-mediated transmembrane currents are able to exert on neuronal excitability ("ceiling depolarization" hypothesis) and response ("shrinking" hypothesis). Although other mechanisms of action, either ionic or non-ionic, may underlie the function of IL-PFC astrocytes in DBS conditions, it remains that these two hypotheses are in good agreement with the results observed in our in vivo experimental conditions. It is likely that the regulation of extracellular K^+ within the mPFC plays actually a key role in this context (Fig. 6). At any rate, our results also highlight the important role played by glia in the stress buffering effects of the behavioral control mediated by coordinated activity of the mPFC–DRN pathway (Maier and Watkins, 2010; Warden et al., 2012).

In conclusion, the present study shows that a glial lesion within the site of stimulation counteracted the beneficial effects of IL-DBS at 130 but not 30 Hz. As translational outcome, it is proposed that an unaltered neuronal/glial system constitutes a major prerequisite to optimize

antidepressant DBS efficacy. It is also suggested that decreasing frequency (e.g., to 30 Hz) could heighten antidepressant response of partial responders and that consuming caffeine might be at risk of weakening the beneficial action of DBS.

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Competing Interests

The authors have declared that no conflict of interest exists.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2015.06.023.

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