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Deep brain stimulation electrode insertion and depression: Patterns of activity and modulation by analgesics

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ABSTRACT

Background: An initial antidepressant effect when using deep brain stimulation (DBS) of the subcallosal area of the cingulate cortex (Cg25) to treat resistant depression that could be the result of electrode insertion has been described. We previously showed that electrode insertion into the infralimbic cortex (ILC; the Cg25 rodent correlate) provokes a temporally limited antidepressant-like effect that is counteracted by non-steroidal anti-inflammatory drugs, such as those routinely used for pain relief.
Objective: We characterized the effect of electrode insertion using functional neuroimaging and evaluated the impact of different analgesics on this effect.
Methods: The effect of electrode insertion into the ILC was evaluated by positron emission tomography. The effect of analgesics (ibuprofen, tramadol and morphine) on the behavioral effect induced by electrode insertion were evaluated through the forced swimming test and the novelty suppressed feeding test. Furthermore, glial fibrillary acidic protein (GFAP) and p11 expression were measured.
Results: Electrode implantation produces an antidepressant- and anxiolytic-like effect, a local decrease in glucose metabolism, and changes in several brain regions commonly related to depression and the antidepressant response. Ibuprofen counteracted the behavioral and molecular changes produced by electrode insertion (changes in GFAP and p11 protein expression). However, analgesics with no anti-inflammatory properties (e.g., tramadol) neither counteract the behavioral effects of electrode implantation nor the molecular mechanisms triggered.
Conclusions: Analgesics without anti-inflammatory properties may not limit the transient benefit produced by electrode insertion reducing the time required to achieve remission in depressive DBS patients.

Abbreviations: ^{18}F-FDG, ^{18}F-fluorodeoxyglucose; ANOVA, analysis of variance; Cg25, subcallosal area of the cingulate cortex; COX, cyclooxygenase; CT, computed tomography; DBS, deep brain stimulation; FWHM, full width at half maximum; GFAP, glial fibrillary acidic protein; ILC, infralimbic cortex; MDD, major depressive disorder; mFST, modified forced swimming test; mPFC, medial prefrontal cortex; MRI, magnetic resonance imaging; NSAIDs, non-steroidal anti-inflammatory drugs; NSFT, novelty-suppressed feeding test; PET, positron emission tomography; qRT-PCR, quantitative reverse transcription polymerase chain reaction; ROI, region of interest; SSRIs, selective serotonin reuptake inhibitors; vmPFC, ventromedial prefrontal cortex; VTA, ventral tegmental area.

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1. Introduction

Deep brain stimulation (DBS) is a surgical technique currently being studied for its application in medically-refractory major depressive disorder (MDD). DBS in the subcallosal area of the cingulate cortex (Cg25) has been proposed as an efficient and safe alternative for patients suffering from resistant MDD [1–3]. Data from some clinical trials studying this possibility suggested that there may be an early beneficial effect related to electrode insertion. Indeed, patients submitted to Cg25-DBS seem to experience a reduction in the severity of their depressive symptoms just after surgery, which was followed by a relapse during the first month of DBS treatment. Subsequently, they recovered gradually until long-term efficacy was achieved [1–3]. Although this phenomenon has yet to be specifically evaluated in patients, we previously found that electrode insertion up to the infralimbic cortex (ILC; the rodent Cg25 correlate) provokes an antidepressant-like effect over a limited period (established after 1 week and lost by 6 weeks), independent of the electrical impulse. Furthermore, this effect was accompanied by an enhancement in reactive astrogliosis, inflammatory mediators and in the expression of the p11 (S100A10) protein in the ventromedial prefrontal cortex (vmPFC). Strikingly, these behavioral and neurochemical changes were abolished by non-steroidal anti-inflammatory drugs (NSAIDs) [4]. In accordance with these data from rodents, a retrospective clinical study showed that the early response of depressed patients to Cg25-DBS was worse when they received NSAIDs in the post-operative period. Thus, it appears that the short-term amelioration of depressive symptoms following DBS surgery might reflect the beneficial effects of local neuroinflammation and as such, the use of NSAIDs may counteract this response in patients subjected to DBS [4]. However, it is unknown if other drugs may achieve pain relief without counteracting these transient benefits.

In this study, the glucose metabolism evoked by electrode implantation into the ILC was evaluated using positron emission tomography (PET). Subsequently, the behavioral and molecular effects of analgesic drugs with different mechanisms of action (ibuprofen, tramadol and morphine) were explored in relation to the benefits produced by electrode insertion during the DBS procedure.

2. Methods and materials

2.1. Animals and experimental design

These studies were carried out on male Wistar rats (University of Cádiz) weighing 250–300 g at the beginning of the experiments. Animals were kept under standard laboratory conditions: 12-h light/dark cycle at a constant temperature (21 ± 1 °C), with food and water available *ad libitum*. All procedures were approved by the Animal Research Ethics Committee of the University of Cádiz and Gregorio Marañón Hospital, and they were performed in accordance with European Guidelines (2010/63/EU) and Spanish Law (RD 53/2013).

These studies were carried out on six independent sets of animals (see experimental design in Fig. S1AF) using naive (unoperated controls) and DBS-*off* animals (rats with electrodes implanted into the ILC). Our previous study showed an antidepressant-like effect one week after implantation of the electrodes and thus, the neuroimaging and behavioral experiments, and tissue extraction were all performed one week after electrode implantation [4]. Firstly, to evaluate the effect of electrode implantation on brain metabolic activity, a set of animals was scanned by positron emission tomography/computed tomography (PET/CT) 45 min after ^{18}F-fluorodeoxyglucose (^{18}F-FDG) injection into the tail vein (n = 8–10 rats per group: Fig. S1A). Secondly, we explored the effect of different analgesics on the antidepressant-like effect of electrode implantation. To this end, naïve animals were tested in the paw-pressure test to search for an effective analgesic dose for each drug used (n = 8 rats per group: Fig. S1B). After dose selection, naïve and DBS-*off* animals were treated with the analgesic or the respective vehicle alone for 9 days, starting 2 days before the surgery. These animals were evaluated in different studies: 1) Locomotor activity was assessed in the open-field test (n = 8–10 rats per group) to ensure that the behaviors observed in the other tests were not due to alterations in spontaneous locomotion (Fig. S1C); 2) Animals (n = 7–8 rats per group) were assessed in the modified forced swimming test (mFST) to explore the effect of treatments on the antidepressant-like effect (four animals from each experimental group were then perfused for immunohistochemistry: Fig. S1D); 3) Animals (n = 8–10 rats per group) were assessed in the novelty-suppressed feeding test (NSFT) in order to explore the possible anxiolytic-like effect of electrode implantation and the effect of the analgesic (Fig. S1E); 4) Animals (n = 6–7 rats per group) were used for quantitative reverse transcription polymerase chain reaction (qRT-PCR) studies (Fig. S1F).

2.2. Drugs

Ibuprofen (50 mg/kg, p.o.: Zambon S.A., Spain), tramadol hydrochloride (8 mg/kg, i.p.: Sigma-Aldrich, Spain) and morphine hydrochloride (1.5 mg/kg, i.p.: Spanish Agency of Medicines and Medical Devices, Spain) were used. Ibuprofen was dissolved in 30% propylene glycol, while tramadol hydrochloride and morphine hydrochloride were diluted in physiological saline. All the drugs were injected once daily for 9 days at 1 ml/kg of body weight under blind conditions. They were administered before each behavioral test and tissue extraction, except for the paw-pressure test in which the drug was administered just once, 30 min before the test. Appropriate vehicle groups were used in each case.

2.3. Surgery

The rats were anesthetized with a ketamine/xylazine mixture (100 mg/kg and 12 mg/kg, i.p.) and placed in the stereotaxic apparatus (Kopf Instruments, mod. 900, CA). Electrodes were implanted bilaterally into the ILC at the following stereotaxic coordinates: +3 mm anterior and ±0.5 mm lateral of bregma lowered 4.8 mm ventral from the duramater [5]. Three small stainless steel screws were also attached to the skull to secure the electrodes with dental cement (Proclinic S.A., Spain). The bipolar stimulating electrodes were two stainless-steel enamel-coated wires (150 μm diameter: California Fine Wire, CA) with 1 mm of exposed surface.

2.4. Imaging studies

PET/CT scans were acquired in naïve rats and DBS-*off* animals. Rats were deprived of food for 6 h before imaging and the animals were scanned using a small-animal PET/CT scanner (ARGUS PET/CT, SEDECAL, Spain) under isoflurane anesthesia (3% induction and 1.5% maintenance in 100% O_2). The ^{18}F-FDG (approximately 1 mCi) was injected into the tail vein and after an uptake period of 45 min, the animals were scanned for 45 min. Images were reconstructed using a 2D-OSEM (2D Ordered Subset Expectation Maximization) algorithm, which claims a spatial resolution for this scanner of 1.45 mm Full Width at Half Maximum (FWHM), with a voxel size of $0.3875 \times 0.3875 \times 0.7750$ mm^3. The energy window was 400–700 keV, and decay and dead-time corrections were applied.

CT studies were acquired using the following parameters: 340 mA, 40 KV, 360 projections, 8 shots, and 200 μm resolution. CT images were reconstructed using a Feldkamp algorithm (isotropic voxel size of 0.121 mm) [6]. All CT studies were co-registered with a random reference CT scan using an automatic rigid registration method and the spatial transformation obtained for each CT image was subsequently applied to the corresponding PET. A brain mask segmented on this magnetic resonance imaging (MRI) study was applied to all registered PET images and the resulting images were smoothed with an isotropic Gaussian filter (2 mm FWHM). Voxel values were normalized to the average white matter intensity in order to obtain the regional characterization of metabolic changes, circumventing the overall differences in animal brain metabolism. A region of interest (ROI) analysis was also performed to determine the intragroup global metabolic differences. Brain and white matter masks segmented on the MRI template were used for this analysis.

2.5. Behavioral tests

2.5.1. Paw-pressure test

The effect of analgesics was measured using the paw-pressure test [7] on naïve animals to demonstrate that the doses of each drug used produced analgesia (Fig. S1B), consistent with previous publications [8,9]. A graded motor-driven device (750-g cut-off: Ugo Basile, Italy) was employed to measure the withdrawal threshold following mechanical hind paw stimulation. Rats received a single administration of each drug 30 min before the test and three measures were taken on each paw at 5 min intervals and the average values were used.

2.5.2. Open-field test

Spontaneous locomotor activity was measured over a 15 min period using an open-field apparatus that consisted of a square plexiglas box (45 × 45 cm) with 35 cm high walls (Fig. S1C). The total distance travelled (arbitrary units) was measured as an indicator of locomotor activity using a S.M.A.R.T system (Spontaneous Motor Activity Recording and Tracking: Panlab, S.L., Spain).

2.5.3. Modified forced swimming test

The mFST is one of the most widely used preclinical tools to study anti-depressant activity in rats (Fig. S1D). Rats were placed individually into a clear Plexiglas cylinder (height, 50 cm; diameter, 20 cm) filled with 30 cm of water at 23 ± 1 °C for two sessions: 15 min pre-test followed by a 5 min test performed 24 h later [10]. The tests were recorded and the predominant behavior (immobility, swimming or climbing) was scored using customized software (Red Mice, Spain). A reduction in immobility in this test was considered to indicate antidepressant activity [10]. Following the mFST, the rats were anesthetized and perfused for the immunohistochemistry studies.

2.5.4. Novelty-suppressed feeding test

This test measures a rodent's aversion to eating in a novel environment as an indicator of anxiety-related activity (Fig. S1E) [11]. The rats were deprived of food and water 24 h before the experiment and the animals were then placed in a strongly lit novel Plexiglas cage (70 × 70 cm square box with 40 cm high walls), which contained a white platform with 4 chow pellets in the center. The latency to eat the food was measured (simply sniffing or touching the pellets was not scored) and the cut-off time was 360s.

2.6. Immunohistochemistry

Coronal sections (30 μm) were processed for free-floating immunohistochemistry against glial fibrillary acidic protein (GFAP) and GFAP expression in the ILC was evaluated by densitometry and quantified using Image J software (NIH, USA) as previously described [4]. .

2.7. Quantitative RT-PCR

Rats were anesthetized and their brains were rapidly dissected and frozen at −80 °C. The *p11* mRNA in the total vmPFC (covering both the prelimbic cortex and ILC) was determined by qRT-PCR. Total RNA was extracted using 300 μl of the TRIzol reagent (Invitrogen, CA) and the RNA samples (1 μg) were treated with RNase-free DNase I (New England Technologies, UK) and used to synthesize cDNAs with the iScript cDNA Synthesis Kit (Bio-Rad, Spain) in a final volume of 20 μl. Real-time PCR was performed in a CFX Connect Real-Time PCR Detection System using iTaq Universal SYBR Green Supermix (Bio-Rad, Spain). The primers used for PCR amplification were: *p11* (designed by using NCBI primer design tool), forward 5′-GGCCCAGGTTTCAACAGATTCTTC-3′ and reverse 5′- CTCAGGTCCTCCTTTGTCAAGT-3'; *Gapdh* [12], forward 5′-AGGTC GGTGTGAACGGATTTG-3′ and reverse 5′-TGTAGACCATGTAGTTGAG GTCA-3'. Samples were assayed in duplicate and the relative gene expression was quantified using the $2^{-\Delta\Delta Ct}$ method [13]. Changes in gene expression were expressed relative to the control samples and normalized to the internal control gene (*Gapdh*).

2.8. Histological verification

To determine the exact position of the electrodes, coronal microtome sections (40 μm) were stained with neutral red solution. Only the animals with the electrodes correctly placed in the ILC were included in the respective analyses (Figs. S2A–B).

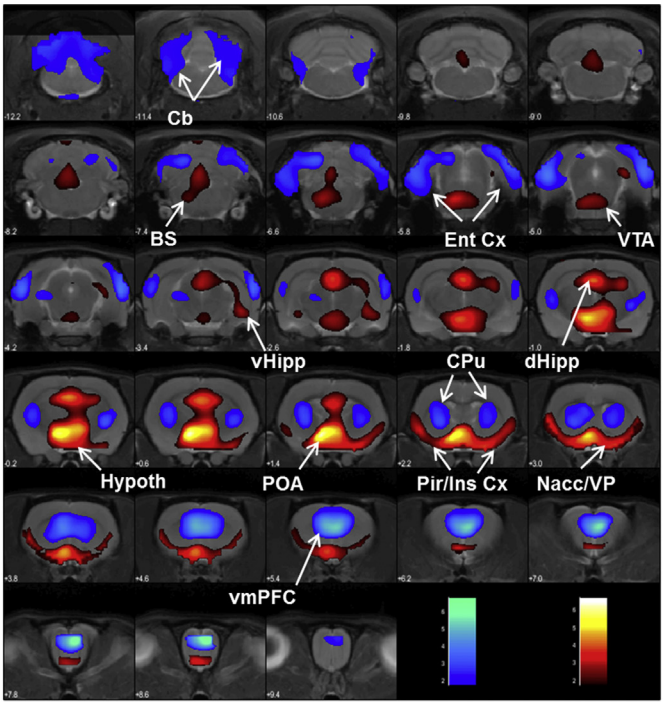
2.9. Data analysis

Statistical analysis of the regional PET data was performed using the SPM12 software package (Statistical Parametric Mapping, Wellcome Trust Center for Neuroimaging: London, UK). The groups were compared by means of an unpaired Student's t-test, uncorrected for multiple comparisons. To reduce type I error, a 50-voxel clustering threshold (spatial-extent) was also applied. The behavioral, immunohistochemical and qRT-PCR data are presented as the mean ± S.E.M., and they were analyzed with Prism 5.0 GraphPad and Statistica 10.0 software. An unpaired Student's *t*-test was used to compare the values from two groups. Comparisons of more than two groups were performed using two-way ANOVA followed by the Bonferroni post-hoc test for multiple comparisons. All p-values < 0.05 are considered significant (see Table S1 for detailed statistical analysis).

3. Results

3.1. Neuroimaging study

Measurements of the global changes in whole brain metabolism did not reveal a significant difference between the naïve (2.72 ± 0.60: mean ± S.D.) and DBS-*off* rats (2.86 ± 0.20, $p > 0.05$). Electrode implantation reduced the metabolic activity in the vmPFC and striatum (right and left: $p < 0.001$), and in the entorhinal cortex and cerebellum (right: $p = 0.001$; left: $p = 0.001$). Conversely, it increased glucose metabolism in the dorsal hippocampus and brainstem relative to the naïve control group, as well as in ventral regions such as the piriform and insular cortex, nucleus accumbens, ventral tegmental area (VTA), ventral pallidum, hypothalamus, preoptic area (right and left: $p < 0.001$) and ventral hippocampus (left: $p = 0.006$; Fig. 1).



	ROI	Voxel size	Side	↑/↓	T	p value	p FWE-corr
Naïve vs DBS-off	Nacc/VP - Pir/Ins Cx - POA Hypoth - dHipp - VTA - BS	2770	R & L	↑	6.68	<0.001	0.029
	vHipp		L	↑	2.93	0.006	0.929
	vmPFC - Cpu	2951	R & L	↓	6.76	<0.001	0.027
	Ent Cx - Cb		L	↓	3.95	0.001	0.547
	Ent Cx - Cb		R	↓	3.93	0.001	0.557

Fig. 1. Effect of electrode implantation into the infralimbic cortex (ILC) on brain metabolism. Colored PET overlays on MR reference indicates an increase in ^{18}F-FDG uptake (hot colors) or a decrease (cold colors) in glucose metabolism for DBS-*off* (electrodes implanted into the ILC and maintained in place for 1 week) relative to naïve (unoperated controls). The table summarizes the differences for the respective regions of interest (ROI) and sides of the brain (right, R; left, L). BS, brainstem; Cb, cerebellum; CPu, caudate putamen nucleus; dHipp, dorsal hippocampus; Ent Cx, entorhinal cortex; Hypoth, hypothalamus; Nacc/VP, nucleus accumbens and ventral pallidum; Pir/Ins Cx, piriform and insular cortex; POA, preoptic area; vHipp, ventral hippocampus; VTA, ventral tegmental area; vmPFC, ventromedial prefrontal cortex (n = 7–8 rats per group). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Behavioral tests

3.2.1. Paw-pressure test and open-field test

Ibuprofen, tramadol and morphine significantly increased the paw withdrawal threshold in naïve animals relative to respective vehicle alone (Fig. S3), demonstrating a clear analgesic effect. Furthermore, none of these analgesics significantly modified the animal's motor activity in the open-field test (Fig. S4).

3.2.2. Modified forced swimming test

The mFST is used to predict antidepressant-like effects and in general, the analgesics alone did not produce an antidepressant-like effect at the doses used in naïve animals (Fig. 2). As described previously [4], electrode implantation induced a significant reduction in the immobility scores of the animals that received the vehicle alone ($p < 0.001$), which was also correlated with an increase in swimming ($p < 0.001$: Fig. 2A–C).

Ibuprofen attenuated the antidepressant-like effect of electrode implantation in the mFST, increasing the DBS-*off* animal's immobility ($p < 0.01$) and diminishing their swimming behavior ($p < 0.001$: Fig. 2A). By contrast, the behavioral score remained unaltered in morphine-treated DBS-*off* animals relative to those that received the vehicle alone (Fig. 2B) and while tramadol did not change the immobility score in DBS-*off* animals, it did significantly reduce the time spent swimming ($p < 0.01$: Fig. 2C). Therefore, neither morphine nor tramadol counteracted the antidepressant-like effect of DBS-*off* animals.

3.2.3. Novelty-suppressed feeding test

The NSFT was used to evaluate whether electrode implantation influenced other aspects of the depressive-like state, such as anxiety. Electrode implantation strongly reduced the latency to feed compared to that of naïve animals ($p < 0.05$), indicative of an anxiolytic-like effect in DBS-*off* animals (Fig. 2D–F). None of the drugs significantly modified the latency to feed of naïve animals (Fig. 2D–F). When it was evaluated whether analgesics attenuated the anxiolytic-like effect produced by electrode insertion, ibuprofen did increase the time to begin eating in DBS-*off* animals relative to those that received the vehicle alone ($p < 0.05$: Fig. 2D). By contrast, neither morphine nor tramadol reduced the latency to feed in the DBS-*off* animals (Fig. 2E–F).

3.3. Reactive gliosis

None of the treatments assessed modified the expression of GFAP in the ILC of naïve animals (Fig. 3A-O). Significantly, electrode implantation in the ILC induced an increase in the density of GFAP-positive cells around the target area of insertion in the animals that

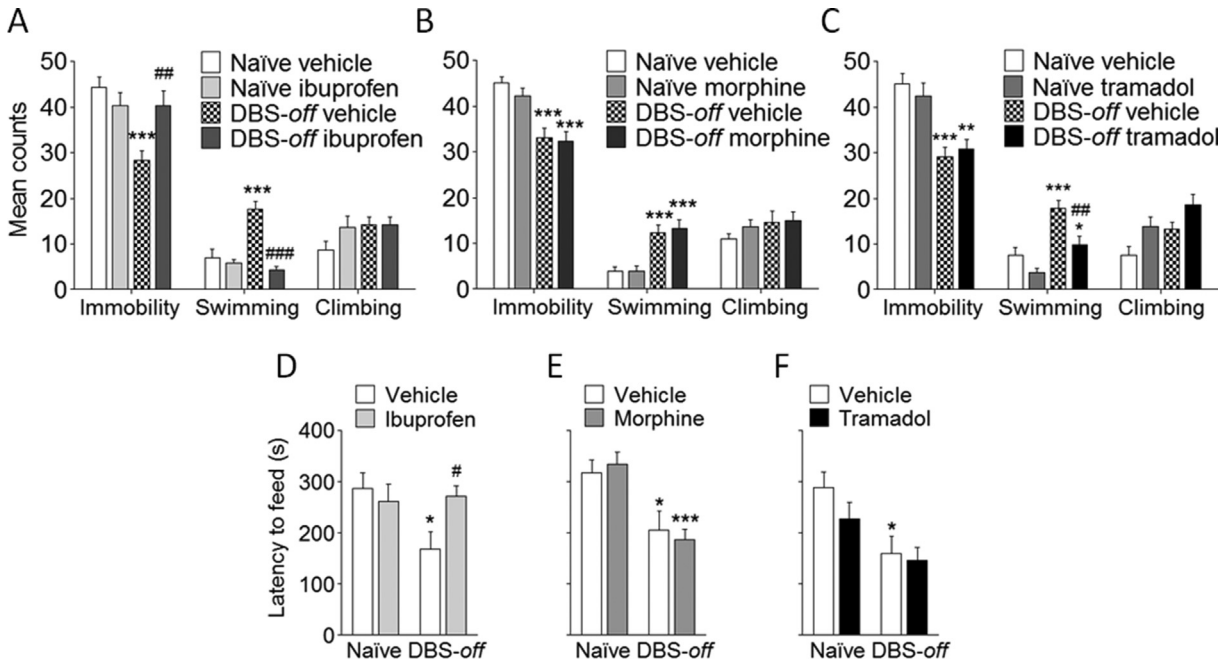


Fig. 2. Effect of analgesics on the antidepressant- and anxiolytic-like effect induced by electrode implantation into the infralimbic cortex (ILC). (A–C) The bar graphs represent the mean total time + S.E.M. in the modified forced swimming test (mFST) spent immobile, swimming and climbing (in arbitrary units). (D–F) The bar graphs represent the mean latency to feed (in seconds) + S.E.M. in the novelty-suppressed feeding test (NSFT). The experimental groups used were naive (unoperated controls) and DBS-*off* (electrodes implanted into the ILC and maintained in place for 1 week). (A,D) Ibuprofen (50 mg/kg), (B,E) morphine (1.5 mg/kg) and (C,F) tramadol (8 mg/kg) was administered once daily for 9 days before performing the behavioral tests. The data was analyzed with a two-way ANOVA followed by a Bonferroni post-hoc test: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to the naïve group; #p < 0.05, ##p < 0.01, ###p < 0.001, with respect to the vehicle-treated group (n = 7–10 rats per group).

received the vehicle alone ($p < 0.05$: Fig. 3). As expected from our previous data indicating that NSAIDs (indomethacin and ibuprofen) counteract the astrogliosis induced by electrode implantation in the ILC [4], ibuprofen pre-treatment diminished the GFAP expression in DBS-*off* animals ($p < 0.01$: Fig. 3A–E). Conversely, the increased density of GFAP-positive cells in the ILC in DBS-*off* animals persisted in animals pre-treated with morphine (Fig. 3F–J) or tramadol (Fig. 3K–O).

3.4. *p11 mRNA*

Electrode implantation significantly augmented the *p11* mRNA levels in the vmPFC of animals that received the vehicle alone ($p < 0.01$: Fig. 4A). In naïve animals, neither ibuprofen nor morphine altered the p11 expression relative to that in animals which received the vehicle alone. By contrast, tramadol did enhance the p11 levels in naïve animals ($p < 0.001$: Fig. 4B). In DBS-*off* animals, ibuprofen reduced ($p < 0.05$), morphine did not change and tramadol enhanced p11 expression relative to the vehicle-treated animals ($p < 0.01$: Fig. 4C).

4. Discussion

DBS electrode insertion alters the patterns of brain activity in areas frequently affected during depression and targeted by antidepressant treatment [14]. The behavioral response to electrode insertion is accompanied by reactive astrogliosis and an increase in *p11* mRNA expression in the area targeted. These processes are attenuated by ibuprofen but not by other analgesics that do not have anti-inflammatory properties (opioids).

Electrode implantation up to the ILC is associated with a decrease in glucose metabolism in the vmPFC after one week, consistent this procedure provoking a transient decrease in neuronal activity in the target and surrounding area that might produce antidepressant-like effects. Probe or electrode implantation has been proposed to induce local inactivation [15,16] and there is also evidence that lower activity in the Cg25 or ILC is associated with an antidepressant response [17–22]. Specifically, a decrease in regional cerebral blood flow and glucose metabolism following Cg25-DBS therapy was correlated with the improvement in depressive symptoms [1,2]. Hence, this early effect may well be produced simply in response to electrode insertion.

Electrode insertion also seems to modulate remote areas implicated in depression or the antidepressant response [14]. Indeed, an increase in glucose metabolism is found in the insular cortex, a region implicated in emotional processing [23–25], as well as in the piriform cortex. A similar effect in the piriform cortex was reported during DBS to the medial prefrontal cortex (mPFC) [26]. The increased activity in the hypothalamus, preoptic area, VTA, nucleus accumbens and ventral pallidum is also of interest, as is the weaker activity in the caudate-putamen nucleus. These findings are linked to abnormalities in the hypothalamic-pituitary-adrenal axis [27] and the reward system [28] that are associated with the pathophysiology of depression [29]. Alternatively, the weak metabolic activity in the cerebellum is consistent with neuroimaging of depressed patients successfully treated with antidepressants [18,30] and with the consequences of mPFC-DBS in rodents [26,31].

In terms of the hippocampus, electrode implantation enhances glucose metabolism in agreement with data about DBS in the mPFC in rodents [26,31]. Previous studies have reported both an increase or decrease metabolic activity in patients with depression [32–34]. Furthermore, the antidepressant response has been related with either an increase or a decrease of hippocampal activity independently of the pre-treated hippocampal activity level [21], suggesting that the antidepressant effects of mPFC-DBS and electrode implantation may involve the regulation of different pathways. In this sense, it is noteworthy that the entorhinal cortex innervates

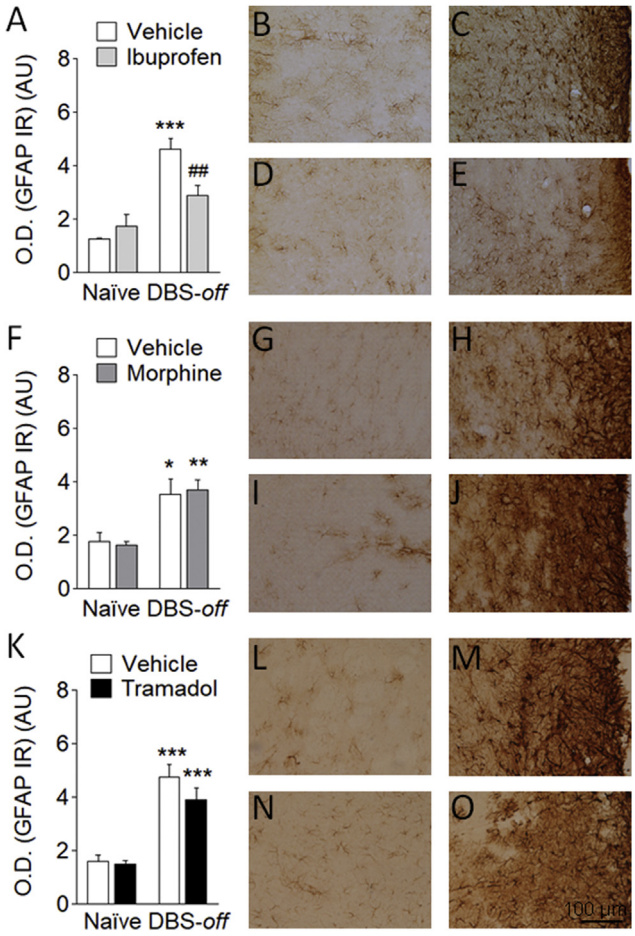


Fig. 3. Effect of analgesics on the expression of GFAP (glial fibrillary acidic protein) in the infralimbic cortex (ILC). The bar graphs represent the mean of the normalized GFAP intensity (arbitrary units) + S.E.M. in naïve (unoperated controls) and DBS-*off* (electrodes implanted into the ILC and maintained in place for 1 week). (A) Effect of ibuprofen (50 mg/kg) on GFAP expression in the ILC. (B–E) Representative images (20X) of GFAP expression in the ILC of vehicle-treated (B) naïve and (C) DBS-*off* animals, and in ibuprofen-treated (D) naïve and (E) DBS-*off* animals. (F) Effect of morphine (1.5 mg/kg) on GFAP expression in the ILC. (G–J) Representative images (20X) of GFAP expression in the ILC of vehicle-treated (G) naïve and (H) DBS-*off* animals, and (I) naïve and (J) DBS-*off* animals pre-treated with morphine. (K) Effect of tramadol (8 mg/kg) on the expression of GFAP in ILC. (L–O) Representative images (20X) of GFAP expression in the ILC of vehicle-treated (L) naïve and (M) DBS-*off* animals, and in tramadol-treated (N) naïve and (O) DBS-*off* animals. All drugs were administrated once daily for 9 days before performing the FST and obtaining the tissue. The data were analyzed by two-way ANOVA followed by a Bonferroni post-hoc test: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to the naïve group; $\#\#p < 0.01$ with respect to the vehicle-treated group (n = 4 rats per group).

inhibitory interneurons of the CA1 [35] and thus, weaker hippocampal input from the entorhinal cortex, the activity of which is also dampened, could influence hippocampal hyperactivity.

Alternatively, inactivation of the ILC increases VTA activity, an effect that is strongly influenced by the activity of the ventral hippocampus [36,37]. This is consistent with our findings since electrode implantation increases the metabolic activity in the VTA, suggesting some coordination of the vmPFC-VTA-ventral hippocampus pathway. Prefrontal cortex exerts a direct control to dopaminergic mesocortical neurons and an indirect control to the mesolimbic pathway by the regulation of the VTA GABAergic neurons [38]. So, it is reasonable to postulate that the hypoactivation of the vmPFC could reduce the activity of the VTA GABAergic interneurons and consequently enhance the activity of the VTA and the limbic nuclei that receive VTA projections, such as the NAc or the hippocampus. Accordingly, it has been recently published the efficacy of DBS on the medial forebrain bundle to treat refractory depression [39,40]. The medial forebrain bundle is the fiber tract that connect the VTA with the limbic system [41]. Additionally, the modulation of the VTA neuronal activity pattern seems to be crucial to reach an antidepressant-like effect by the vmPFC stimulation and to induce adaptive changes in the hippocampus [42,43]. So, electrode insertion could produce an activation of the VTA and the medial forebrain bundle that may be underpinning the beneficial early response observed. Finally, another intriguing finding is the higher activity found in the brainstem, in contrast to the decrease in brainstem activity detected by neuroimaging in response to selective serotonin reuptake inhibitors (SSRIs) [44] or mPFC-DBS in rodents [26,31]. Overall, neuroimaging suggests that electrode insertion into the ILC provokes changes at local and distant brain areas related with depression.

Changes in glucose metabolism are accompanied by behavioral and molecular changes. This study confirms our previous data regarding the antidepressant-like effect of electrode insertion in the mFST [4], extending these findings to also show a clear anxiolytic-like effect in the NSFT. These behavioral effects are accompanied by reactive astrogliosis and an increase in *p11* mRNA expression in the area targeted. Growing evidence implicates glial anomalies in several cortical areas in the pathophysiology of depression and antidepressant treatment activates GFAP in the mPFC in preclinical models [45,46]. Thus, the activation of glia in response to electrode implantation is likely to contribute to the behavioral effects reported, as witnessed through the increase in cells containing GFAP detected here. Conversely, local brain damage activates repair mechanisms that will probably induce an antidepressant effect for a limited period of time. It is known that p11 is related to immune system regulation and participates in the inflammatory processes [47]. Accordingly, reactive astrocytes upregulate p11 expression following a brain lesion [48]. In relation with depression, a weaker p11 protein expression has been associated with the pathophysiology of depression both in humans and in rodent models [49]. Furthermore, several antidepressant therapies augment the p11 detected in the frontal cortex of rodents [50–52] and while more p11 protein was found previously in the insertion area [4], we now show an increase in *p11* mRNA that seems to be crucial for the antidepressant effect.

We also evaluated the effect of analgesic drugs on the insertion related effects of DBS as the antidepressant response can be blocked by NSAIDs (indomethacin and ibuprofen) [4]. Ibuprofen blocks cyclooxygenase (COX) signaling and as a result, it probably reduces the astrogliosis induced by traumatic brain injury [53], potentially abolishing the antidepressant-like and anxiolytic-like effect of electrode insertion. These events are consistent with our previous data from animal models and with a worse prognosis of depressed patients subjected to DBS who take NSAIDs postoperatively [4]. There is evidence from mice and humans that anti-inflammatory drugs attenuate the antidepressant effect of SSRIs [52]. Indeed, these drugs are thought to selectively increase the levels of certain cytokines and of p11 in the prefrontal cortex, and these molecular changes are antagonized by NSAIDs [52]. However, it is important to note that several evidences linked depression with a chronic and general activation of the inflammatory system [54] and it has been pointed that several antidepressants drugs decrease the peripheral expression of some inflammatory mediators [55]. So, these data suggest that antidepressant drugs could produce opposite effects at cortical and systemic level. Electrode implantation in the vmPFC would produce a local and transient activation of the inflammatory system in a discrete brain region but not a chronic immune dysregulation.

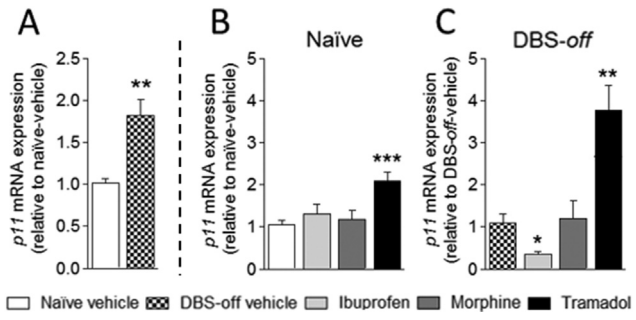


Fig. 4. The effect of analgesics on p11 expression in the ventromedial prefrontal cortex (vmPFC). The bar graphs represent the mean + S.E.M. of the normalized p11 expression relative to the control animals (relative units) in the naïve (unoperated controls) and DBS-*off* rats (electrodes implanted into the infralimbic cortex (ILC) and maintained in place for 1 week). (A) Effect of electrode implantation in vehicle-treated animals. (B) The effect of ibuprofen (50 mg/kg), morphine (1.5 mg/kg), tramadol (8 mg/kg) in naïve animals, and (C) the effect of the administration of these drugs to DBS-*off* animals relative to the vehicle-treated group. All the drugs were administrated once daily for 9 days before tissue extraction. The data were analyzed with a T-student test: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to the control group (n = 6–7 rats per group).

Given that NSAIDs block the early antidepressant response, the effect of other analgesic drugs with a distinct mechanism of action was evaluated (morphine and tramadol). Morphine is primarily an agonist of mu-opioid receptors, whereas tramadol inhibits serotonin and noradrenaline reuptake, in addition to binding to mu-opioid receptors [56]. We showed that the antidepressant- and anxiolytic-like effect of electrode implantation remains unaltered when combined with morphine or tramadol treatment. Importantly, none of these activities are the result of changes in locomotor activity and moreover, morphine and tramadol do not modify GFAP expression in the ILC. It has been reported that opioids rapidly and significantly increase astrocyte and macrophage/microglial activation in several brain areas, such as the prefrontal cortex [57–59]. However, opioid administration did not increase GFAP expression in the vmPFC here. Perhaps opioid treatment helps sustain gliosis in the target area and in turn, the antidepressant- and anxiolytic-like effect provoked. Alternatively, analgesic opioids do not counteract the elevated p11 expression in the vmPFC that results from electrode insertion. Conversely, tramadol increases the p11 expressed in both naïve and DBS-*off* animals, which could be related to the pro-antidepressant-like effects of tramadol reported at higher doses [60–62]. Indeed, tramadol did not significantly modify FST behavior and there was a clear trend towards increased climbing in both naïve and DBS-*off* animals. Therefore, the mechanisms underlying the antidepressant action of tramadol could be related to the increase in p11 expression in the vmPFC.

5. Conclusions

In summary, DBS electrode insertion leads to robust changes in the brain and the administration of NSAIDs may interfere with the early antidepressant response provoked. Analgesic drugs with different mechanisms of action (e.g., tramadol) could relieve perioperative pain without affecting the initial antidepressant effect. However, given that DBS involves major surgery a positive balance must be struck between the benefits and adverse effects in patients subjected to DBS therapy. Specific clinical studies, prospective or retrospective, should be performed to determine the impact of analgesics on the effects of DBS in mental illness.

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Statement of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.brs.2018.06.010.

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