Molecular Psychiatry (2014) 19, 607–614

www.nature.com/mp

ORIGINAL ARTICLE

Early responses to deep brain stimulation in depression are modulated by anti-inflammatory drugs

L Perez-Caballero[1,2], R Pérez-Egea[2,3], C Romero-Grimaldi[2,4], D Puigdemont[2,3], J Molet[5], J-R Caso[2,6], J-A Mico[1,2], V Pérez[2,3], J-C Leza[2,7] and E Berrocoso[2,8]

Deep brain stimulation (DBS) in the subgenual cingulated gyrus (SCG) is a promising new technique that may provide sustained remission in resistant major depressive disorder (MDD). Initial studies reported a significant early improvement in patients, followed by a decline within the first month of treatment, an unexpected phenomenon attributed to potential placebo effects or a physiological response to probe insertion that remains poorly understood. Here we characterized the behavioural antidepressant-like effect of DBS in the rat medial prefrontal cortex, focusing on modifications to rodent SCG correlate (prelimbic and infralimbic (IL) cortex). In addition, we evaluated the early outcome of DBS in the SCG of eight patients with resistant MDD involved in a clinical trial. We found similar antidepressant-like effects in rats implanted with electrodes, irrespective of whether they received electrical brain stimulation or not. This effect was due to regional inflammation, as it was temporally correlated with an increase of glial-fibrillary-acidic-protein immunoreactivity, and it was blocked by anti-inflammatory drugs. Indeed, inflammatory mediators and neuronal p11 expression also changed. Furthermore, a retrospective study indicated that the early response of MDD patients subjected to DBS was poorer when they received anti-inflammatory drugs. Our study demonstrates that electrode implantation up to the IL cortex is sufficient to produce an antidepressant-like effect of a similar magnitude to that observed in rats receiving brain stimulation. Moreover, both preclinical and clinical findings suggest that the use of anti-inflammatory drugs after electrode implantation may attenuate the early anti-depressive response in patients who are subjected to DBS.

Molecular Psychiatry (2014) **19,** 607–614; doi:10.1038/mp.2013.63; published online 28 May 2013

Keywords: deep brain stimulation; depression; non-steroidal anti-inflammatory drugs; subgenual cingulated gyrus

INTRODUCTION

Deep brain stimulation (DBS) in the subgenual cingulated gyrus (SCG) is a promising non-pharmacological therapeutic alternative to treat resistant major depressive disorder (MDD).[1–3] Interestingly, these studies also described an initial and unexpected short-term antidepressant effect. This effect was described as a biphasic effect with a large initial improvement in the first week, followed by a decline and a subsequent progressive improvement that reached a plateau after 6 months.[2] This transient early amelioration of symptoms has also been described in other diseases where DBS has been employed,[4–7] and it has been attributed to a possible placebo effect. However, an alternative physiological cause has also been proposed, referred to as an 'insertional effect', involving local responses to the introduction of the probe into the neural tissue.[8] It is plausible that such neurophysiological changes could account for the early antidepressant effects of DBS and that these transient benefits could reduce the time required to reach remission.

To address this question, we characterized the antidepressant-like effect of the DBS procedure in the medial prefrontal cortex (PFC) of rats, which covers the prelimbic (PL) and infralimbic (IL) cortex, and that represents the rodent region homologous to the human SCG (Brodmann areas (BA) 24/25).[9,10] In addition, we evaluated the early phase of DBS therapy in a controlled clinical trial of patients diagnosed with treatment-resistant MDD. Our data suggest that early DBS response is induced by local inflammation, which is supported by the fact that anti-inflammatory drugs used peri-operatively counteract this beneficial effect.

MATERIALS AND METHODS

Animals

Male Wistar rats (University of Cádiz, weighing 200–250 g at the beginning of the experiments) were maintained under standard laboratory conditions. Animal handling and use was in accordance with the European Commission's directive (2010/63/EU) and Spanish Law (RD-1201/2005). See Figure 1 legend and Supplementary Figure S1 for description of every experimental group and design.

Surgery and stimulation procedure

Once anaesthetized (100 mg kg^{-1} ketamine, 12 mg kg^{-1} xylazine, intra-peritoneal), the rats were placed in a stereotaxic apparatus for surgery. The stimulation target chosen was the IL cortex (AP + 3 from bregma, L ± 0.5 from the midline suture, DV 4.8 mm from the dura mater). In another set of

[1]Neuropsychopharmacology and Psychobiology Research Group, Department of Neuroscience (Pharmacology and Psychiatry), University of Cadiz, Cadiz, Spain; [2]Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Madrid, Spain; [3]Department of Psychiatry, Hospital de la Santa Creu i Sant Pau, Institut d'Investigació Biomèdica Sant Pau (IIB-Sant Pau), Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; [4]Neuropsychopharmacology and Psychobiology Research Group, *Salus Infirmorum* Faculty of Nursing, University of Cadiz, Cadiz, Spain; [5]Department of Neurosurgery, Hospital de la Santa Creu i Sant Pau, Institut d'Investigació Biomèdica Sant Pau (IIB-Sant Pau), Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; [6]Department of Psychiatry, Faculty of Medicine, University Complutense and Instituto de Investigación 12 de octubre, Madrid, Spain; [7]Department of Pharmacology, Faculty of Medicine, Universidad Complutense, Madrid, Spain and [8]Neuropsychopharmacology and Psychobiology Research Group, Psychobiology Area, Department of Psychology, University of Cadiz, Cadiz, Spain. Correspondence: Dr E Berrocoso, Department of Psychology, Campus Universitario Río San Pedro s/n, Puerto Real (Cádiz) 11510, Spain.
E-mail: esther.berrocoso@uca.es
Received 27 June 2012; revised 6 April 2013; accepted 15 April 2013; published online 28 May 2013

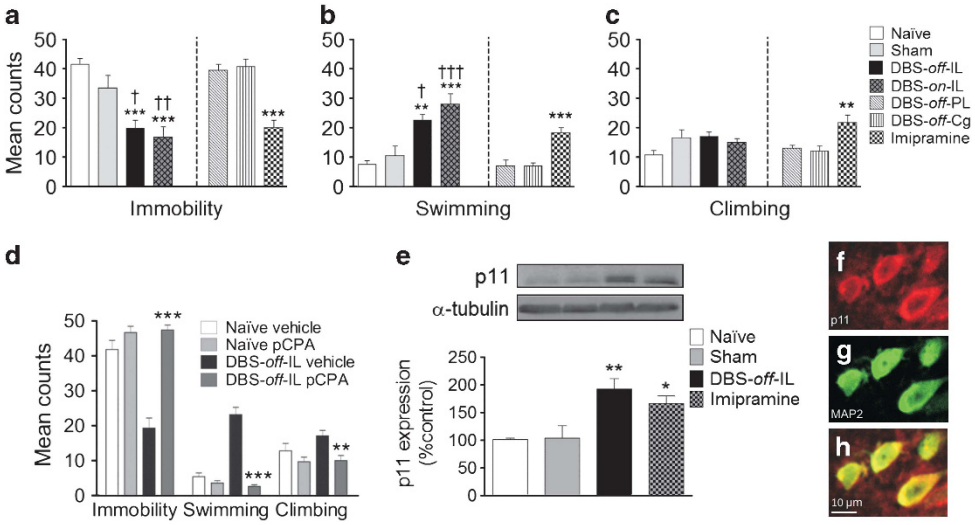


Figure 1. (**a**–**c**) Effect of electrode insertion and deep brain stimulation (DBS) in the medial prefrontal cortex on the forced swimming test (FST) in rats 1 week after surgery. Graph represents (**a**) immobility, (**b**) swimming and (**c**) climbing behaviours during the FST. Experimental groups were: naive (no surgical or pharmacological manipulation), sham (electrodes inserted up to the infralimbic (IL) cortex and immediately removed), DBS-*off*-IL (electrodes implanted in the IL cortex and kept in place but no stimulation was delivered), DBS-*on*-IL (electrical stimulation in the IL cortex delivered for 4 h after the pre-test and 2 h before the test), DBS-*off*-PL (electrodes implanted in the prelimbic (PL) cortex but no electrical stimulation delivered), DBS-*off*-Cg (electrodes implanted in the cingulate (Cg) cortex but no electrical stimulation delivered) and imipramine (15 mg kg^{-1} intraperitoneal (i.p.), administered 23, 19 and 2 h before the test). (**d**) Role of the serotonergic system on electrode implantation in the FST. para-Chlorophenyl-alanine methyl ester (pCPA, 100 mg kg^{-1}, i.p.) was administered once daily on 3 consecutive days before the test. (**e**) Western blots probed for p11 protein in the PL and IL cortex in naive, sham, DBS-*off*-IL and imipramine-treated rats (15 mg kg^{-1}, i.p., once a day for 14 days). Representative bands of p11 and α-tubulin are shown above each bar. (**f**–**h**) Magnified view of p11 (**f**, red) and microtubule-associated protein 2 (MAP2) (**g**, green) immunoreactivity in DBS-*off*-IL rats (**h**, co-localization in merged images in yellow). See Supplementary Figures S1A and B for a schematic illustration of every experimental design. The values are means + s.e.m. One or two-way analysis of variance followed by Bonferroni's test or Student's *t*-test: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus naive or respective vehicle-treated rats; $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$ and $^{\dagger\dagger\dagger}P<0.001$ versus sham rats ($n=7$–10 for the FST, $n=4$ for immunohistochemistry and $n=6$ rats for western blotting techniques).

experiments, the electrodes were implanted in the PL cortex (AP + 3.2, L ± 0.5, DV 3 mm) and the cingulate cortex (AP + 2.7, L ± 0.5, DV 1.3 mm) (Supplementary Figure S2).[11] The bipolar stimulating electrodes were two stainless-steel enamel-coated wires (California Fine Wire, Grover Beach, CA, USA) with 1 mm of exposed surface. The stimulation protocol consisted of monophasic square wave pulses (100 μA, 130 Hz and 90 μs).[12]

Forced swimming test (FST)

FST was performed in large plastic cylinders filled with water for a period of 15 min in the first swimming session (pre-test) and again 24 h later for 5 min under the same conditions (test).[13] Behaviour (immobility, swimming or climbing) was assessed at 5-s intervals throughout the duration of the test session using customized software (Red-Mice, Cadiz, Spain). After FST, rats were killed and studied by western blotting, enzyme-linked immunosorbent assay or immunohistochemistry.

Open field test

Locomotor activity was measured using an open field apparatus (45 × 45 × 35 cm^3). Total distance travelled was measured over testing period using S.M.A.R.T (Spontaneous Motor Activity Recording and Tracking: Panlab, S.L., Barcelona, Spain).

Chronic unpredictable stress (CUS)

One group of animals was subjected to CUS for a period of 5 consecutive weeks (Figure 4a). Four weeks after the beginning of the CUS, the animals were split into two groups: naive or DBS-*off*-IL (electrodes implanted and kept in place but no stimulation was delivered). During the entire experiment, No_CUS animals were housed in a separate room. Preference for sucrose solutions over water was monitored on a weekly basis. Furthermore, the animals were also assessed in the FST.

Western blotting

Rats were killed by decapitation, and the PL and IL cortex were removed bilaterally. Equal amounts of protein from tissue homogenates were separated in SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) gels and transferred to polyvinylidene difluoride membranes. All samples were incubated with the primary antibody (mouse anti-p11, 1:500; Abcam, Cambridge, UK; rabbit anti-cyclooxygenase-1 (COX1), -2 (COX2), -tumour necrosis factor receptor type 1 (TNFR1) and 2 (TNFR2), 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Membranes were washed and incubated with corresponding horseradish peroxidase-conjugated secondary antibody (1:10000 for p11 and 1:3000 for the rest of proteins). Antibody binding was detected by enhanced chemiluminescence, and appropriate film expositions were acquired with Versadoc 5000 (Bio-Rad Laboratories, Madrid, Spain) and were quantified with Image J Software (NIH, Bethesda, MD, USA). The protein levels were normalized using α-tubulin or β-actin.

Enzyme-linked immunosorbent assay

Levels of TNFα, interleukin-1β (IL-1β), interleukin-6 (IL-6) and interferon-γ were measured in the tissue homogenates from the PL and IL cortex using commercially available kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions.

Immunohistochemistry

Coronal brain sections (30 μm) were first incubated with a rabbit antibody against glial-fibrillary-acidic-protein (anti-GFAP, 1:2000; DAKO, Glostrup, Denmark) and later with a biotinylated secondary antibody (Jackson Immuno Research Laboratories, West Grove, PA, USA). Immunodetection was achieved using an ABC kit and by reaction with 3,3-diaminobenzidine tetrahydrochloride.

To evaluate p11 expression, sections were incubated with the two primary antibodies: chicken anti-p11 (1:200; Abcam) and mouse against

microtubule-associated protein 2 (anti-MAP2, 1:200; Millipore, Billerica, MA, USA) or rabbit anti-GFAP (1:2000; DAKO). The sections were washed and then incubated with the secondary antibodies: anti-chicken Alexa-Fluor-568, anti-mouse Alexa-Fluor-633 and anti-rabbit Alexa-Fluor-488 (1:1000; Invitrogen S.A., Madrid, Spain). The omission of primary antibodies resulted in no detectable staining in optical immunodetection studies, although some non-specific fluorescence in the electrode area (typically 80 μm around the injury) was observed. Therefore, this area was excluded in the analysis of fluorescence images.

Image analysis

GFAP expression was evaluated by densitometry with an Olympus-BX60 (Olympus, Hamburg, Germany) microscope under homogeneously lit bright-field illumination, digitalized using an Olympus-DP71 digital camera and Cell F software (Olympus). Photomicrographs were taken at $\times 20$ magnification and converted into 8-bit images using Image J software (NIH). Grey levels were then converted to optical densities and the mean grey values of IL cortex per section was calculated using the following formula: (ROI mean grey-GBLR mean grey)/(ULR mean grey-GBLR mean grey); where 'ROI' = region of interest, 'GBLR' = glass background labelling region and 'ULR' = unspecific labelling region). In each group, values of GFAP expression were expressed as the mean grey value of densitometry/section. The co-localization of p11 and MAP2 or GFAP immunolabeling was evaluated using a Leica Spectra Confocal microscope (Leica Microsystems GmbH, Wetzlar, Germany). Each multi-fluorophore image was separated into individual images and converted into 8-bit images.

Drugs

Imipramine, para-Chlorophenyl-alanine methyl ester (pCPA) and indomethacin were obtained from Sigma-Aldrich (St Louis, MO, USA) and ibuprofen from Zambón S.A. (Barcelona, Spain).

Human studies

Subjects were recruited in Santa Creu i Sant Pau Hospital (Barcelona, Spain), and they were fully informed of the aims and risks of the study. The patient group comprised of eight patients (six female/two male) with refractory MDD according to DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revision) criteria (clinical and demographic characteristics and inclusion/exclusion criteria are described in[14]). Depressive symptoms were rated according to the17-item Hamilton Depression Rating Scale (HDRS-17). Patients were defined as responders if they exhibited a decrease of at least 50% in their HDRS-17 score. Remission was considered to be achieved in patients with a score of $\leqslant 8$ in the HDRS-17 scale.

DBS electrodes were implanted in the white matter adjacent to the SCG region (BA 24/25) using a Leksell-G stereotactic frame (Elekta Instruments, Stockholm, Sweden). Quadripolar electrodes (Model-3387; Medtronic, Minneapolis, MN, USA) were implanted bilaterally, guided by an intraoperative stereotactic magnetic resonance image and stereotactic cerebral computer tomography. The leads were connected to a programmable internal capsule pulse generator (Medtronic). Chronic stimulation began within 48 h after surgery in all the patients. Stimulating parameters (3.5–5 V, 135 Hz, 120–210 μs) were adjusted during subsequent visits if the patient failed to show improvement.

Statistics

The data were expressed as the mean ± s.e.m. in animal studies and mean ± s.d. in clinical studies. The statistical significance was assessed using an unpaired Student's *t*-test, one- or two-way or repeated-measures analysis of variance followed by a Bonferroni's test (Supplementary Table S1). Significance was defined as $P<0.05$.

RESULTS

Effect of DBS in the FST

The antidepressant-like effect of DBS was first evaluated 1 week after surgery (Figures 1a–c and Supplementary Figure S1A). FST analysis revealed a significant decrease in immobility in DBS-*on*-IL when compared with naive and sham ($F_{(3,36)}=14.15$, $P<0.001$ and $P<0.01$, respectively), accompanied by an increase in swimming ($F_{(3,36)}=14.54$, $P<0.001$ in each case). Strikingly, similar results were found in the DBS-*off*-IL group in the absence of stimulation. Indeed, animals implanted with electrodes exhibited significant differences in immobility and swimming ($P<0.001$ versus naive and $P<0.05$ versus sham). Furthermore, no differences were detected in locomotor activity between groups, precluding a motor component in the observed effects in the FST (Supplementary Figure S3).

Whether the results observed were specific to a given brain area was determined by implanting the electrode into the PL and cingulate cortex, which produced no antidepressant-like effect in either case (PL: immobility $t=0.10$, $df=12$; swimming $t=0.35$, $df=12$; climbing $t=0.11$, $df=12$; cingulate: immobility $t=0.48$, $df=13$; swimming $t=0.47$, $df=13$; climbing $t=0.29$, $df=13$, $P>0.05$, respectively; Figures 1a–c and Supplementary Figure S2). As expected, imipramine decreased immobility ($t=6.57$, $df=18$, $P<0.001$) and enhanced swimming and climbing ($t=-5.15$, $df=18$, $P<0.001$; $t=-3.32$, $df=18$, $P<0.01$, respectively) (Figures 1a–c). Overall, these findings demonstrate that electrode placement as deep as the IL cortex exerts an antidepressant-like effect in the FST.

Next, as electrode implantation (DBS-*off*-IL) induced an increase in swimming, animals were pre-treated with pCPA to determine whether this antidepressant-like activity was mediated by effects on 5-hydroxytryptamine (5-HT) transmission. Pre-treatment with pCPA increased immobility and diminished the active behaviours in the DBS-*off*-IL (Figure 1d and Supplementary Figure S1B). Furthermore, as elevating the amount of p11 in the PFC seems to be essential for selective serotonin reuptake inhibitors to exert their antidepressant action,[15,16] we evaluated p11 expression in the PL and IL cortex (Figures 1e–h). The amount of p11 increased significantly in DBS-*off*-IL as compared with naive ($F_{(3,20)}=9.10$, $P<0.01$) and, as expected, after imipramine treatment ($P<0.05$). Furthermore, co-immunolabeling showed that p11 specifically co-localized with MAP2 (neuronal marker) and that p11 expression was visibly higher in DBS-*off*-IL animals at a distance of 500–700 μm from the site of injury (Supplementary Figures S4 and S5). No co-localization was seen with GFAP (astrocyte marker).

Temporal effect of electrode implantation

Independent groups of animals were evaluated in the FST 1, 2 or 6 weeks after surgery (Figures 2a–c and Supplementary Figure S1C). Results showed that the antidepressant-like effect in DBS-*off*-IL was conserved for 2 weeks after surgery, as witnessed by a decrease in immobility and an increase in swimming when compared with naive ($P<0.001$ in each case). However, these effects were absent 6 weeks after the implantation of electrodes. Therefore, it is plausible that the inflammatory response produced by surgery is implicated in the antidepressant-like response.[8] Immunohistochemical analysis revealed an increase in GFAP expression in the IL cortex in DBS-*off*-IL 1 and 2 weeks after surgery when compared with naive ($P<0.001$ and $P<0.01$, respectively) and sham-operated rats ($P<0.001$ and $P<0.05$, respectively). Furthermore, such an increase was not found after 6 weeks of implantation (Figures 2d–m).

Additionally, we also explored the effect of electrode insertion soon after surgery (Supplementary Figure S6). Forty-eight hours after surgery, DBS stimulation was able to produce an antidepressant-like effect compared with naive (DBS-*on*-IL; $P<0.05$). However, electrode insertion (DBS-*off*-IL) does not have such an effect.

Non-steroidal anti-inflammatory drugs (NSAIDs) influence the antidepressant-like effect of probe implantation

To determine whether preventing the inflammatory response could counteract the antidepressant-like effect seen 1 week after electrode implantation, animals were pre-treated with indomethacin or ibuprofen (Figure 3a, Supplementary Figures S1D, S7A and

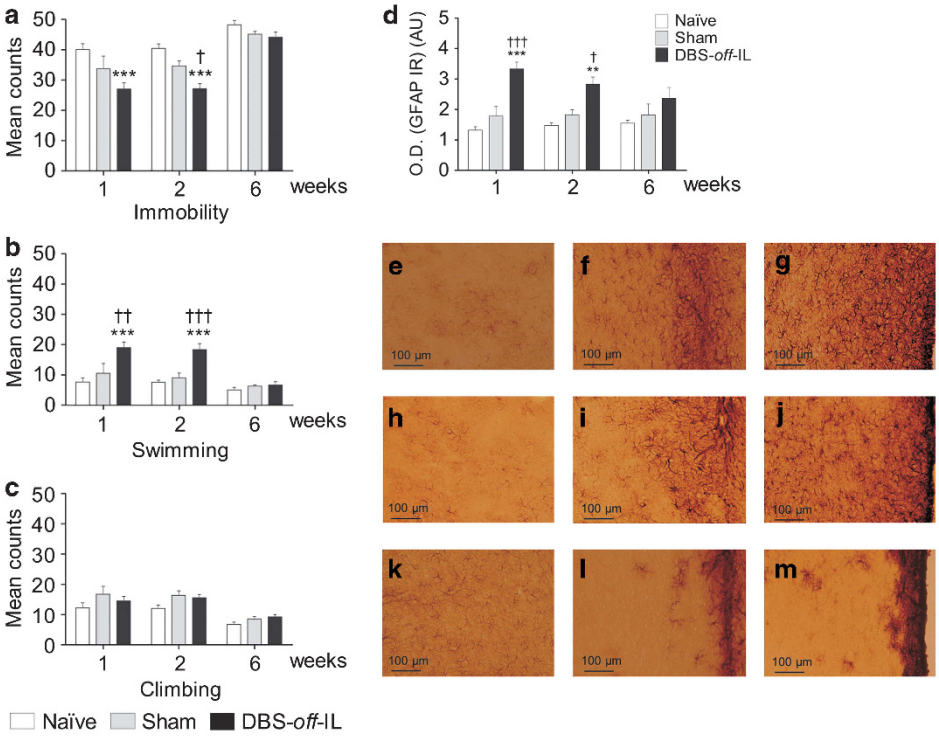


Figure 2. (**a**–**c**) Effects of electrode implantation on the forced swimming test (FST) over time. Rats were tested 1, 2 and 6 weeks after surgery and the graph represents (**a**) immobility, (**b**) swimming and (**c**) climbing behaviour. (**d**) Expression of glial-fibrillary-acidic-protein (GFAP) in the infralimbic (IL) cortex, a representative area of the microlesion, 1, 2 and 6 weeks after electrode implantation. Graph represents the normalized GFAP intensity (arbitrary units (AU)). (**e**–**m**) Representative images (20 ×) of GFAP expression in the area of electrode insertion 1 week after electrode implantation in (**e**) naive, (**f**) sham and (**g**) DBS-*off*-IL rats, 2 week after surgery in (**h**) naive, (**i**) sham and (**j**) DBS-*off*-IL rats and 6 weeks post-implantation in (**k**) naive, (**l**) sham and (**m**) DBS-*off*-IL rats. The following experimental groups were used: naive (no surgical or pharmacological manipulation), sham (electrodes were inserted into the target area and immediately removed) and DBS-*off*-IL (electrodes were implanted and kept in place, but no stimulation delivered). See Supplementary Figure S1C for a schematic illustration of the experimental design. Values are means + s.e.m., Two-way analysis of variance followed by a Bonferroni's test: $^{**}P<0.01$, $^{***}P<0.001$ versus respective naive group; $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$ versus respective sham group ($n=7$–10 for the FST and $n=4$ for immunohistochemistry). DBS, deep brain stimulation; O.D., optical density.

7B). Both increased immobility ($P<0.01$) and diminished swimming ($P<0.001$) in DBS-*off*-IL animals that were administered with these drugs. Furthermore, NSAIDs significantly decreased the density of GFAP-positive cells in DBS-*off*-IL (indomethacin group: $P<0.001$ and ibuprofen group: $P<0.05$; Figures 3b–f and Supplementary Figures S7C–G). As expected, higher levels of inflammatory mediators (cytokines: IL-1β: $P<0.001$ and TNFα: $P<0.01$) and COX1 and COX2 enzymes ($P<0.001$ both) were found in the DBS-*off*-IL group (Figures 3g–h). However, the pre-treatment with indomethacin significantly attenuated the amounts of TNFα ($P<0.05$), as well as COX expression (COX1: $P<0.01$ and COX2: $P<0.001$). No changes were found in the levels of other inflammatory cytokines (IL-6 and interferon-γ) or in the expression of TNFR1 and TNFR2. Furthermore, p11 expression was also diminished by indomethacin pre-treatment ($P<0.001$; Figure 3h).

Effect of electrode implantation in the CUS

The antidepressant-like effect of DBS-*off*-IL was evaluated in animals submitted to CUS (Figure 4a), an animal model of depression. A decline in the preference for sucrose solutions over water is considered an index of anhedonic-like behavior, which was already noticeable between stressed and non-stressed rats 1 week after CUS. This difference became particularly pronounced after 4 weeks of stress, at which point the electrodes were implanted. In line with previous data,[17] electrode implantation in the stressed group led to an increase in sucrose preference in the first week (80% DBS-*off*-IL CUS versus 59% naive CUS; $P<0.05$) (Figure 4b). In the FST, naive CUS significantly displayed greater immobility ($P<0.05$) and less swimming ($P<0.05$) than the control (naive No_CUS), whereas, as expected, electrode implantation decreased immobility ($P<0.05$) and increased swimming activity ($P<0.01$). Interestingly, the implantation of electrodes in the stressed group (DBS-*off*-IL CUS) caused a decrease in immobility ($P<0.001$) and an increase in both active movements relative to the naive animals (Figure 4c).

The effect of NSAIDs in the early effect of DBS in MDD patients

One week after implantation, all patients showed clear improvements in their symptoms.[14] However, a general reversion was observed soon after (Figure 5a), as previously reported.[2,3] Successful implantation in SCG was confirmed,[14] and no significant differences in the HDRS-17 response were detected between the patients who received electrical stimulation in BA24 or BA25 1 month after surgery ($t=2.5$, $df=5$, $P>0.05$).

Based on our findings in rats, we retrospectively evaluated the post-operative regime of analgesic/anti-inflammatory drug administration in relation to the HDRS-17 score. Patients were split into two groups: the first group included patients who received anti-inflammatory drugs (for pain control) during the first month after surgery, while those in the second group received no treatment (Figures 5b and c). Although all patients received bilateral DBS, similar effects were only observed 1 week post implantation, after which divergent effects were detected between the two groups of

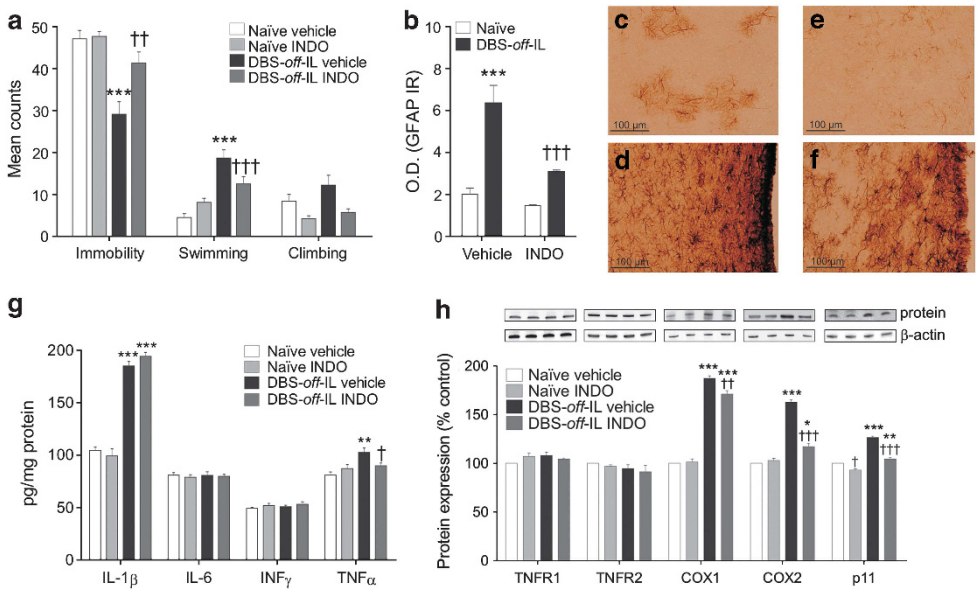


Figure 3. (**a**) Effect of treatment with the non-steroidal anti-inflammatory indomethacin (INDO) on the antidepressant-like effect on the forced swimming test (FST). Graph represents immobility, swimming and climbing in the FST. (**b**) Effect of INDO treatment on the expression of glial-fibrillary-acidic-protein (GFAP) in the infralimbic (IL) cortex, a representative area of the microlesion. Graph represents normalized GFAP intensity (arbitrary units). (**c**–**f**) Representative images (20 ×) of GFAP expression in the IL cortex of vehicle-treated (**c**) naive and (**d**) DBS-*off*-IL, (**e**) naive and (**f**) DBS-*off*-IL rats pre-treated with INDO. (**g**) Effect of INDO treatment on the expression of interleukin-1β (IL-1β), IL-6, interferon-γ (IFNγ) and tumour necrosis factor-α (TNFα) in the PL and IL cortex by enzyme-linked immunosorbent assay (ELISA). (**h**) Effect of INDO treatment on the expression of TNF receptor type 1 (TNFR1) and 2 (TNFR2), cyclooxygenase-1 (COX1) and -2 (COX2) and p11 protein in the PL and IL cortex by western blotting. Representative bands of each protein are shown above each bar. INDO (1 mg kg^{-1}, intraperitoneal) was administered once daily for 9 days before FST and tissue extraction. The following experimental groups were used: naive (no surgical or pharmacological manipulation) and DBS-*off*-IL (electrodes were implanted and kept in place, but no stimulation delivered). Electrodes were implanted 7 days before testing. See Supplementary Figure S1D for a schematic illustration of the experimental design. Graph bars represent mean + s.e.m. Two-way analysis of variance followed by Bonferroni's test: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus respective naive group; $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$ versus respective vehicle ($n=8$–10 for the FST, $n=4$ for immunohistochemistry and $n=6$ rats for ELISA and western blotting techniques). DBS, deep brain stimulation; O.D., optical density.

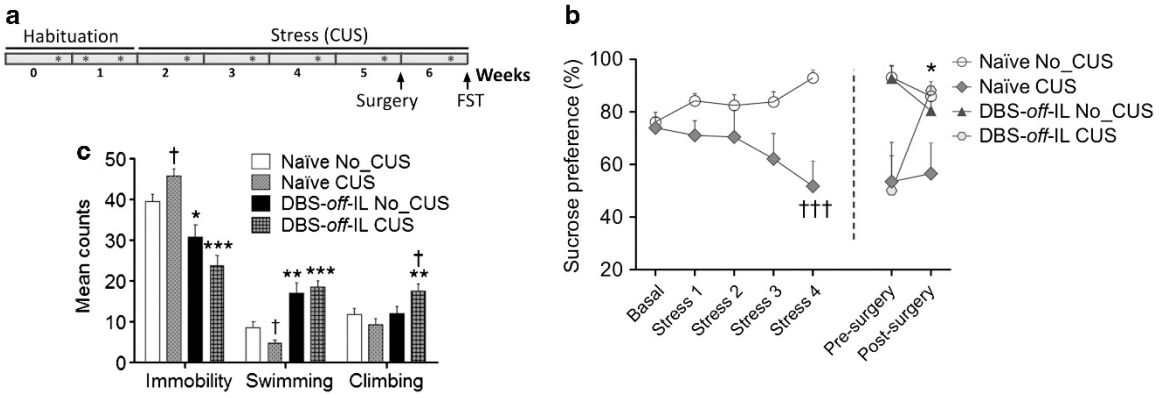
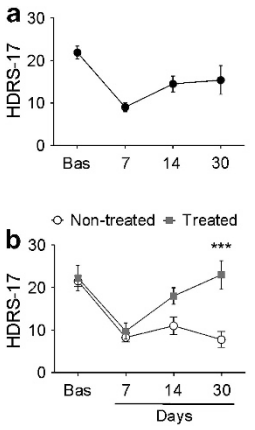


Figure 4. (**a**) Schematic representation of chronic unpredictable stress (CUS) procedure. Animals weighing 300 g were housed individually at the beginning of the habituation period. The CUS group was subjected to several stressors during 5 weeks, whereas animals of No_CUS group remained undisturbed. Stress regime involved: food or water deprivation, 45° cage tilt, intermittent illumination, soiled cage, paired housing, stroboscopic illumination (150 flashes min^{-1}) and periods of no stress. All stressful situations lasted for 10–14 h, and they were applied individually and continuously, day and night. The asterisk indicates sucrose preference (SP) test. The day of surgery (after 4 weeks of CUS) the animals were split between naive (no surgical or pharmacological manipulation) or DBS-*off*-IL groups (electrodes implanted in the infralimbic (IL) cortex and kept in place but no stimulation was delivered). The forced swimming test (FST) was conducted 1 week after the surgery. (**b**) The effect of the CUS and the surgery in the SP test. Rats were initially trained to drink a sucrose solution during the habituation period with a two-bottle choice procedure (one containing 1% sucrose solution and the other containing water) for 1 h. The consumption of water or sucrose was measured by weighing the bottles. The position of the two bottles was varied randomly from trial to trial. SP was calculated as SP (%) = (sucrose intake (g)/(sucrose intake (g) + water intake (g)) × 100. The animals were deprived of water and food 23 h before the test. During the CUS period, the rats were evaluated once a week with the same procedure. (**c**) The FST was conducted at the end of the experiment. Graphs represent mean + s.e.m. Two-way analysis of variance followed by Bonferroni's test: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus respective naive group; $^{\dagger}P<0.05$, $^{\dagger\dagger\dagger}P<0.001$ versus respective No_CUS group ($n=8$–11 per group). DBS, deep brain stimulation.

patients. The four patients who did not receive analgesic/anti-inflammatory exhibited a dramatic reduction in HDRS scores 1 week after implantation, with all patients reaching response criterion and two of the four patients achieving remission. Strikingly, this state was conserved over the entire month, with three of the four patients meeting the remission criterion at 4



c

	Bas	7 days	14 days	30 days	Treatment
No treatment					
Patient 1	24	10	12	13	-
Patient 6	22	10	10	5	-
Patient 7	18	6	6	5	-
Patient 8	22	7	16	8	-
Mean ± SD	21,50 ± 1,2	8,25 ± 1,0	11,00 ± 2,0	7,75 ± 1,9	
Analgesic-anti-inflamatory treatment					
Patient 2	19	7	17	32	Acetaminophen (0-3 days PS, headache)
Patient 3	21	7	22	24	Ibuprofen (0-5 days PS, headache)
Patient 4	18	10	20	19	Ibuprofen (0-30 days PS, migraine and myalgia)
Patient 5	31	15	13	17	Metamizole (7-17 days PS, headache)
Mean ± SD	22,25 ± 2,9	9,75 ± 1,9	18,00 ± 1,9	23,00 ± 3,3	

Figure 5. (**a**, **b**) Patient outcomes measured by the 17-item Hamilton Depression Rating Scale (HDRS-17) for 4 weeks after electrode implantation, grouped according to post-surgery analgesic/anti-inflammatory treatment. All patients received deep brain stimulation during this period. (**c**) Treatment duration and reason for prescription for each patient. Oral doses were as follows: acetaminophen, 500 mg/3 times a day; non-steroidal anti-inflammatory drugs: ibuprofen, 600 mg/3 times per day, and metamizole, 575 mg/3 times a day, PS: days post surgery. Graphs represent mean ± s.d. Two-way repeated-measures analysis of variance followed by Bonferroni's test: ***$P<0.001$ versus untreated patients ($n=4$ patients per group).

weeks. Almost all the patients who received analgesic/anti-inflammatory drugs reached the response criterion after 1 week (3/4), with two of the four patients achieving remission. However, the response deteriorated 2 weeks after surgery, with just one of the four patients meeting the response criteria. Accordingly, the antidepressant efficacy fell substantially over the month, with none of the patients responding by 4 weeks.

DISCUSSION

This translational study demonstrates that the procedure of implanting an electrode into the IL cortex is sufficient to produce benefits in animal models of antidepressant activity. These findings are consistent with the initial effects observed in a series of patients treated for 1 month with DBS. Overall suggest that this early antidepressant effect of DBS is the result of an inflammatory 'insertional effect' provoked by electrode implantation, as it can be blocked by pre-treatment with NSAIDs.

We show here that the mere implantation of electrodes produces an antidepressant-like effect in the FST. This effect was of a similar magnitude to that observed after high frequency stimulation or treatment with imipramine, and analogous effects were found in the CUS model of depression. The electrode's trajectory involves the entire medial PFC, including the IL, PL and cingulate cortex. However, our results show that the electrodes need to be inserted up to the IL to produce an antidepressant-like effect. Indeed, pharmacological inactivation of the IL cortex induces an antidepressant-like effect in the same model used here,[18,19] and interestingly, the IL cortex and/or the most ventral part of the PL cortex are thought to be the target for the effect of DBS in animal models of depression.[12,17,20,21] Hence, further studies to map the 'insertional effect' and its area of influence would appear to be necessary. Importantly, our findings in animals are consistent with those of a clinical trial in which all patients exhibited a dramatic improvement 1 week after electrode implantation in BA24/25.[14]

The integrity of the 5-HT system seems to be essential for the antidepressant nature of this 'insertional effect'. Analysis of active movements in the FST suggested that serotonergic neurotransmission was involved in the antidepressant-like effect, as corroborated by the decrease in active behaviour in DBS-*off*-IL animals following pCPA administration. Indeed, there is increasing evidence that antidepressant therapies (tricyclic antidepressants, selective serotonin reuptake inhibitors and electroconvulsive therapy) enhance p11 expression, which upregulates the number of 5-HT_{1B} and/or 5-HT_4 receptors at the cell membrane.[15,22] This increase in receptor expression influences the efficacy of serotonergic neurotransmission, leading to antidepressant effects.[23] Indeed, when we assessed p11, we found that electrode implantation also increased p11 levels in DBS-*off*-IL animals, typically in neurons. Moreover, 5-HT_{1B} and 5-HT_4 receptors are localized in several brain regions linked with depression, and particularly, 5-HT_{1B} in the cortex.[24] Thus, it seems plausible that electrode insertion would promote p11 expression, which would, in turn, recruit 5-HT_{1B} receptors and produce antidepressant-like effects. Indeed, it would be of interest to examine 5-HT_{1B} and/or 5-HT_4 receptor co-expression with p11 in the PFC and other brain regions in DBS-*off*-IL animals. Interestingly, electrode insertion into depressed animals (DBS-*off*-IL CUS) increased both swimming and climbing in the FST, suggesting that catecholaminergic neurotransmission, as well as the serotonergic system, may be involved in the antidepressant-like response of depressed animals. Furthermore, the reduced immobility and increased climbing of DBS-*on*-IL forty-eight hours post surgery suggest that distinct mechanisms are involved in the 'insertional effect' and in the effects of the electrical stimulation.

An important question that deserves attention involves the mechanisms by which the 'insertional effect' produces its benefits. In general, the CNS responds to injury by activating several short-term repair mechanisms, and thus, it is plausible that these mechanisms might be involved in the antidepressant-like effects observed. In agreement with our hypothesis (Supplementary Figure S8), intracranial electrode implantation represents an obvious insult that is reflected by transient and local glial activation (reactive astrogliosis: GFAP), which provokes an antidepressant-like effect 1 week later. Accordingly, gliosis was self-limited and coincided temporally with the gradual loss of the antidepressant effect (6 weeks). There is growing evidence that GFAP expression in the PFC is reduced by depression[25–30] and that GFAP activation occurs in response to electroconvulsive[31,32] and pharmacological treatment.[25,33,34] Thus, the temporal transformation of glia into activated (or reactive) states may lead to the expression of inflammatory mediators that would induce p11, increasing serotonergic neurotransmission and leading to the antidepressant effects observed. Not only does p11 traffic 5-HT receptors to the cell membrane but it also participates in the

transient reaction to nervous system lesion,[35,36] and data from cell lines confirm that p11 is regulated by inflammatory molecules like Toll-like receptor-4, nitric oxide and phospolipase A2.[37–39] Furthermore, p11 expression was also enhanced in DBS-*off*-IL animals, and it was more strongly expressed close to the insertion site, consistent with an injury-related effect. Once tissue is repaired (homeostasis), p11 levels would decrease and the antidepressant effect will be lost. Moreover, indomethacin counteracts TNFα production and COX expression, and it also blocks the increase in p11 and the antidepressant-like effect, linking local inflammation with the behavioral response. TNFα might be an important participant in this process, because its administration produces an antidepressant-like effect through a p11-dependent mechanism at PFC.[16] This could reflect a direct effect of TNFα, or it may alternatively occur due to the indirect production of neurotrophic factors.[40–42] Neurotrophic factors, such as brain-derived neurotrophic factor and nerve growth factor, seems to be relevant for antidepressant-like effects in animal models,[43] and they also induce p11 expression.[44–46] Therefore, p11 production might be induced directly or indirectly by a specific mediator produced by tissue injury, leading to an antidepressant effect. Consequently, p11 blockade by NSAIDs would limit this effect, consistent with data from animal and human studies. Furthermore, our findings do not contradict the idea that brain inflammation might provoke depression.[47,48] Depression has been suggested as a process of chronic and general inflammation, chronic and unsuccessful immune dysregulation that persists over time. By contrast, electrode insertion causes acute and local inflammation, that is, an active process associated with successful host defense which is limited in time.[49,50] Thus, local injury in a discrete brain area would promote the activation of mechanisms leading to short-term antidepressant effects.

It is important to note the temporal differences in the effects observed in the animal and clinical studies. We propose that preventive treatment with NSAIDs in rats counteracts the processes triggered by electrode implantation. By contrast, patients received analgesic/anti-inflammatory treatment *ad libitum* and post surgery, and while the initial inflammatory process appears to have occurred in all patients, it was later compromised by the analgesic/anti-inflammatory treatment administered. Importantly, the DBS trial in humans was an open-label design in which all patients received stimulation. Future double-blind trials with groups of stimulated and unstimulated patients will provide a better indication of the effects of DBS and further clarify the role of electrode implantation in the associated antidepressant effects. In addition, the existence of a disease other than MDD (a painful condition) that requires NSAIDs treatment may exacerbate depression and/or hinder early efficacy, and this should also be controlled. Overall, the balance between the benefits and risks of using NSAIDs in MDD patients undergoing DBS should be considered. Furthermore, given the magnitude of this type of surgery, other post-operative analgesic options should be explored.

In summary, our rat studies demonstrate that electrode implantation into the IL cortex is sufficient to produce an early antidepressant-like effect, probably through temporary regional inflammation. We suggest that, in addition to the beneficial effect of DBS, there is another effect that also contributes to the early antidepressant response. Based on our clinical findings, we propose that anti-inflammatory drugs routinely used in a preventive and palliative capacity in DBS surgery may counteract the initial antidepressant effects of this approach.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by grants from the Spanish Ministry of Health; CIBERSAM G18, G19, G12 and G21, 'Proyecto Intramural P91' and PI10/01221, PI06/0662, PS09/00580, PI12/00915, CTS-7748, CTS-510, CTS-4303, FP7-PEOPLE-2010-RG (268377) and SAF2007-62378. We thank JM Lopez-Cepero and A Adell for helpful discussions. We also thank J Gallego-Gamo, R Rey-Brea and JA García-Partida for their excellent technical assistance in the animal study and J Berrocoso for his "Red Mice" software design.

REFERENCES

1 Hamani C, Mayberg H, Snyder B, Giacobbe P, Kennedy S, Lozano AM. Deep brain stimulation of the subcallosal cingulate gyrus for depression: anatomical location of active contacts in clinical responders and a suggested guideline for targeting. *J Neurosurg* 2009; **111**: 1209–1215.
2 Lozano AM, Mayberg HS, Giacobbe P, Hamani C, Craddock RC, Kennedy SH. Subcallosal cingulate gyrus deep brain stimulation for treatment-resistant depression. *Biol Psychiatry* 2008; **64**: 461–467.
3 Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C *et al.* Deep brain stimulation for treatment-resistant depression. *Neuron* 2005; **45**: 651–660.
4 Greenberg BD, Malone DA, Friehs GM, Rezai AR, Kubu CS, Malloy PF *et al.* Three-year outcomes in deep brain stimulation for highly resistant obsessive-compulsive disorder. *Neuropsychopharmacology* 2006; **31**: 2384–2393.
5 Mallet L, Polosan M, Jaafari N, Baup N, Welter ML, Fontaine D *et al.* Subthalamic nucleus stimulation in severe obsessive-compulsive disorder. *N Engl J Med* 2008; **359**: 2121–2134.
6 Maltete D, Chastan N, Derrey S, Debono B, Gerardin E, Lefaucheur R *et al.* Microsubthalamotomy effect at day 3: screening for determinants. *Mov Disord* 2009; **24**: 286–289.
7 Holtzheimer PE, Kelley ME, Gross RE, Filkowski MM, Garlow SJ, Barrocas A *et al.* Subcallosal cingulate deep brain stimulation for treatment-resistant unipolar and bipolar depression. *Arch Gen Psychiatry* 2012; **69**: 150–158.
8 Hirshler YK, Polat U, Biegon A. Intracranial electrode implantation produces regional neuroinflammation and memory deficits in rats. *Exp Neurol* 2010; **222**: 42–50.
9 Ongur D, Price JL. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cereb Cortex* 2000; **10**: 206–219.
10 Takagishi M, Chiba T. Efferent projections of the infralimbic (area 25) region of the medial prefrontal cortex in the rat: an anterograde tracer PHA-L study. *Brain Res* 1991; **566**: 26–39.
11 Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. Compact6th edn. Academic Press: San Diego, CA, USA, 2009.
12 Hamani C, Diwan M, Macedo CE, Brandao ML, Shumake J, Gonzalez-Lima F *et al.* Antidepressant-like effects of medial prefrontal cortex deep brain stimulation in rats. *Biol Psychiatry* 2010; **67**: 117–124.
13 Alba-Delgado C, Llorca-Torralba M, Horrillo I, Ortega JE, Mico JA, Sanchez-Blazquez P *et al.* Chronic pain leads to concomitant noradrenergic impairment and mood disorders. *Biol Psychiatry* 2013; **73**: 54–62.
14 Puigdemont D, Perez-Egea R, Portella MJ, Molet J, de Diego-Adelino J, Gironell A *et al.* Deep brain stimulation of the subcallosal cingulate gyrus: further evidence in treatment-resistant major depression. *Int J Neuropsychopharmacol* 2011; **22**: 1–13.
15 Svenningsson P, Chergui K, Rachleff I, Flajolet M, Zhang X, El Yacoubi M *et al.* Alterations in 5-HT1B receptor function by p11 in depression-like states. *Science* 2006; **311**: 77–80.
16 Warner-Schmidt JL, Vanover KE, Chen EY, Marshall JJ, Greengard P. Antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) are attenuated by antiinflammatory drugs in mice and humans. *Proc Natl Acad Sci USA* 2011; **108**: 9262–9267.
17 Hamani C, Machado DC, Hipolide DC, Dubiela FP, Suchecki D, Macedo CE *et al.* Deep brain stimulation reverses anhedonic-like behavior in a chronic model of depression: role of serotonin and brain derived neurotrophic factor. *Biol Psychiatry* 2012; **71**: 30–35.
18 Scopinho AA, Scopinho M, Lisboa SF, Correa FM, Guimaraes FS, Joca SR. Acute reversible inactivation of the ventral medial prefrontal cortex induces antidepressant-like effects in rats. *Behav Brain Res* 2010; **214**: 437–442.
19 Slattery DA, Neumann I, Cryan JF. Transient inactivation of the infralimbic cortex induces antidepressant-like effects in the rat. *J Psychopharmacol* 2010; **25**: 1295–1303.
20 Gersner R, Toth E, Isserles M, Zangen A. Site-specific antidepressant effects of repeated subconvulsive electrical stimulation: potential role of brain-derived neurotrophic factor. *Biol Psychiatry* 2010; **67**: 125–132.
21 Hamani C, Diwan M, Isabella S, Lozano AM, Nobrega JN. Effects of different stimulation parameters on the antidepressant-like response of medial prefrontal cortex deep brain stimulation in rats. *J Psychiatr Res* 2010; **44**: 683–687.

22 Warner-Schmidt JL, Flajolet M, Maller A, Chen EY, Qi H, Svenningsson P *et al.* Role of p11 in cellular and behavioral effects of 5-HT4 receptor stimulation. *J Neurosci* 2009; **29**: 1937–1946.
23 Svenningsson P, Greengard P. p11 (S100A10)--an inducible adaptor protein that modulates neuronal functions. *Curr Opin Pharmacol* 2007; **7**: 27–32.
24 Egeland M, Warner-Schmidt J, Greengard P, Svenningsson P. Co-expression of serotonin 5-HT(1B) and 5-HT(4) receptors in p11 containing cells in cerebral cortex, hippocampus, caudate-putamen and cerebellum. *Neuropharmacology* 2011; **61**: 442–450.
25 Banasr M, Chowdhury GM, Terwilliger R, Newton SS, Duman RS, Behar KL *et al.* Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Mol Psychiatry* 2010; **15**: 501–511.
26 Banasr M, Duman RS. Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol Psychiatry* 2008; **64**: 863–870.
27 Gosselin RD, Gibney S, O'Malley D, Dinan TG, Cryan JF. Region specific decrease in glial fibrillary acidic protein immunoreactivity in the brain of a rat model of depression. *Neuroscience* 2009; **159**: 915–925.
28 Miguel-Hidalgo JJ, Baucom C, Dilley G, Overholser JC, Meltzer HY, Stockmeier CA *et al.* Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. *Biol Psychiatry* 2000; **48**: 861–873.
29 Ongur D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc Natl Acad Sci USA* 1998; **95**: 13290–13295.
30 Webster MJ, O'Grady J, Kleinman JE, Weickert CS. Glial fibrillary acidic protein mRNA levels in the cingulate cortex of individuals with depression, bipolar disorder and schizophrenia. *Neuroscience* 2005; **133**: 453–461.
31 Jansson L, Wennstrom M, Johanson A, Tingstrom A. Glial cell activation in response to electroconvulsive seizures. *Prog Neuropsychopharmacol Biol Psychiatry* 2009; **33**: 1119–1128.
32 Kragh J, Bolwig TG, Woldbye DP, Jorgensen OS. Electroconvulsive shock and lidocaine-induced seizures in the rat activate astrocytes as measured by glial fibrillary acidic protein. *Biol Psychiatry* 1993; **33**: 794–800.
33 Czeh B, Simon M, Schmelting B, Hiemke C, Fuchs E. Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology* 2006; **31**: 1616–1626.
34 Liu Q, Li B, Zhu HY, Wang YQ, Yu J, Wu GC. Clomipramine treatment reversed the glial pathology in a chronic unpredictable stress-induced rat model of depression. *Eur Neuropsychopharmacol* 2009; **19**: 796–805.
35 De Leon M, Van Eldik LJ, Shooter EM. Differential regulation of S100 beta and mRNAs coding for S100-like proteins (42A and 42C) during development and after lesion of rat sciatic nerve. *J Neurosci Res* 1991; **29**: 155–162.
36 Gveric D, Herrera BM, Cuzner ML. tPA receptors and the fibrinolytic response in multiple sclerosis lesions. *Am J Pathol* 2005; **166**: 1143–1151.
37 Wu T, Angus CW, Yao XL, Logun C, Shelhamer JH. P11, a unique member of the S100 family of calcium-binding proteins, interacts with and inhibits the activity of the 85-kDa cytosolic phospholipase A2. *J Biol Chem* 1997; **272**: 17145–17153.
38 Pawliczak R, Cowan MJ, Huang X, Nanavaty UB, Alsaaty S, Logun C *et al.* p11 expression in human bronchial epithelial cells is increased by nitric oxide in a cGMP-dependent pathway involving protein kinase G activation. *J Biol Chem* 2001; **276**: 44613–44621.
39 Swisher JF, Burton N, Bacot SM, Vogel SN, Feldman GM. Annexin A2 tetramer activates human and murine macrophages through TLR4. *Blood* 2010; **115**: 549–558.
40 Bayas A, Hummel V, Kallmann BA, Karch C, Toyka KV, Rieckmann P. Human cerebral endothelial cells are a potential source for bioactive BDNF. *Cytokine* 2002; **19**: 55–58.
41 Kuno R, Yoshida Y, Nitta A, Nabeshima T, Wang J, Sonobe Y *et al.* The role of TNF-alpha and its receptors in the production of NGF and GDNF by astrocytes. *Brain Res* 2006; **1116**: 12–18.
42 Saha RN, Liu X, Pahan K. Up-regulation of BDNF in astrocytes by TNF-alpha: a case for the neuroprotective role of cytokine. *J Neuroimmune Pharmacol* 2006; **1**: 212–222.
43 Neto FL, Borges G, Torres-Sanchez S, Mico JA, Berrocoso E. Neurotrophins role in depression neurobiology: a review of basic and clinical evidence. *Curr Neuropharmacol* 2011; **9**: 530–552.
44 Okuse K, Malik-Hall M, Baker MD, Poon WY, Kong H, Chao MV *et al.* Annexin II light chain regulates sensory neuron-specific sodium channel expression. *Nature* 2002; **417**: 653–656.
45 Warner-Schmidt JL, Chen EY, Zhang X, Marshall JJ, Morozov A, Svenningsson P *et al.* A role for p11 in the antidepressant action of brain-derived neurotrophic factor. *Biol Psychiatry* 2010; **68**: 528–535.
46 Masiakowski P, Shooter EM. Nerve growth factor induces the genes for two proteins related to a family of calcium-binding proteins in PC12 cells. *Proc Natl Acad Sci USA* 1988; **85**: 1277–1281.
47 Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 2009; **65**: 732–741.
48 Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* 2006; **27**: 24–31.
49 Buffo A, Rite I, Tripathi P, Lepier A, Colak D, Horn AP *et al.* Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain. *Proc Natl Acad Sci USA* 2008; **105**: 3581–3586.
50 Vedam-Mai V, van Battum EY, Kamphuis W, Feenstra MG, Denys D, Reynolds BA *et al.* Deep brain stimulation and the role of astrocytes. *Mol Psychiatry* 2012; **17**: 124–131115.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)