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Deep brain stimulation in treatment-resistant depression in mice: Comparison with the CRF_1 antagonist, SSR125543

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ABSTRACT

Deep brain stimulation (DBS) has been demonstrated to represent a targeted therapeutic alternative for treatment-resistant depression. In this study, we used the unpredictable chronic mild stress (UCMS) test to validate high-frequency electrical stimulation of the cingulate cortex (CC) as a possible treatment to improve behavioral symptoms associated with a depressive-like state in treatment-resistant mice. The effects of DBS were compared with those of the CRF_1 antagonist, SSR125543. Mice were subjected to UCMS, which consisted of the sequential and unpredictable application of mild stressors for a total of 8 weeks. From week 4 until the end of week 6, mice received either a saline injection or were treated with the antidepressant, fluoxetine (10 mg/kg, i.p.). At the end of week 6, fluoxetine-treated mice were subdivided into two populations, that is one responding to fluoxetine, and one not responding, based on their fur coat state, an index of depressive-like state in this test. Non-responders were subsequently subjected to bilateral DBS (at 80 or 120 Hz, 1-h/day) or were treated with SSR125543 (20 mg/kg, i.p.) for two weeks. Stimulation of the CC at 120 Hz in treatment-resistant mice resulted in a normalization of motivated-like responses, anxiety-related behaviors, hyperactivity and aggressiveness. SSR125543 improved motivated-like and aggressive behaviors. These findings demonstrate that bilateral DBS of the CC and, to a lesser extent, pharmacological blockade of the CRF_1 receptor in treatment-resistant mice can attenuate several aspects of depressive-like behaviors, suggesting further that these approaches may represent valid alternatives for the treatment of drug-resistant depressed and/or anxious patients.

1. Introduction

Major depressive disorder (MDD) is a widespread and costly illness, with a prevalence of about 10% worldwide. There are a variety of treatment options available, but a significant proportion of patients do not achieve sustained symptomatic remission. Current antidepressants have limited therapeutic efficacy and it has been conservatively estimated that 10%–30% of patients are treatment-resistant despite multiple treatment attempts (Nierenberg and Amsterdam, 1990; Rush et al., 2006). Deep brain stimulation (DBS), which is successfully used in patients with Parkinson's disease has recently been suggested to represent a possible therapeutic strategy for treatment-resistant depression (TRD) (Shah et al., 2010). The first published report described a clinically significant antidepressant response in patients with TRD after 6 months of open-label bilateral DBS applied to the subcallosal cingulate gyrus (SCG) (Mayberg et al., 2005).

The basis for selection of the SCG, including Brodmann area (BA) 25, originates primarily from functional imaging studies that revealed that patients with depression have an abnormal metabolic pattern in the SCG, often characterized by an increased activity (Mayberg et al., 1999). The SCG is an integral part of the limbic system, which is involved with emotion formation and processing, learning and memory, core behaviors altered in depression. Low mood and antidepressant treatment have consistently been shown to involve the SGC, suggesting a critical role for this region in modulating negative mood states (Mayberg et al., 1999). Structural and ultrastructural changes, neuronal density or hypermetabolism have been reported in the SCG of depressed patients (for a review, see Price and Drevets, 2009). For example, prominent volumetric abnormality has been observed in the SGC of MDD patients, in particular a reduction in gray matter (Drevets et al., 1997). Furthermore, during the depressed phase of bipolar disorder, glucose metabolism in posterior cingulate cortex is abnormally elevated and hemodynamic responses to rewarding or emotional stimuli are altered (Drevets et al., 2002). With this rationale, DBS of the SGC was applied successfully in TRD patients (Kennedy et al., 2011; Lozano et al., 2012; Mayberg et al., 2005). The initial premise was that high-frequency stimulation could disrupt pathological activity and reverse the abnormal metabolic pattern observed in depression. This initial study was replicated in open-label trials of SCG DBS for 12 months in patients with TRD (Lozano et al., 2012). It is noteworthy that other targets for DBS in TRD have been proposed, including the inferior

Abbreviations: DBS, deep brain stimulation; CC, cingulate cortex; UCMS, unpredictable chronic mild stress; MDD, major depressive disorder; SGC, subcallosal cingulate gyrus; TRD, treatment-resistant depression.

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thalamic peduncle, the ventral capsule/ventral striatum, the nucleus accumbens and the habenula (Anderson et al., 2012; Hamani and Nobrega, 2010).

Several recent studies in rats have described the consequences of electrical stimulation of the lateral habenula or the ventromedial prefrontal cortex using the chronic mild stress model (Hamani and Nobrega, 2010; Hamani et al., 2012; Meng et al., 2011) or the forced-swimming test of depression (Hamani et al., 2010a,b). They showed that intermittent or continuous DBS was associated with an improvement in depressive-like symptoms. Moreover, in Wistar-Kyoto rats, which are known to exhibit a depressive-like phenotype, electrical stimulation of the nucleus accumbens led to a decrease in anxiety-like behaviors (Falowski et al., 2011). It is important to note that the animals used in these studies have not been tested for their sensitivity to antidepressants, so that it is not known whether they were drug-resistant or not. It is unclear if similar results would have been obtained in treatment-resistant animals. Thus, studying the impact of DBS in a preclinical model of TRD is important as it would mimic more closely research in human using DBS in depressed patients.

In this context, the objective of this study was to test whether repeated DBS of the cingulate cortex (CC) may be able to improve behaviors reminiscent of certain aspects of human depression in mice selected for their insensitivity to the antidepressant, fluoxetine. Mice were subjected to the unpredictable chronic mild stress (UCMS) procedure, which has been proposed as a naturalistic model of depression, in that it satisfies some criteria for face, predictive, and construct validity (Cryan and Holmes, 2005; Willner, 1997). The CC was selected as a relevant target region because we have observed in a previous study that UCMS exposure produced profound changes in gene expression in this region, effects that were reversed by chronic treatment with fluoxetine (Surget et al., 2008b). The effects of DBS were compared to those of the CRF_1 receptor antagonist, SSR125543 (Gully et al., 2002), based on the idea that the blockade of this receptor may be a possible therapeutic strategy in treatment-resistant depressed patients (Griebel and Holsboer, 2012; Surget and Belzung, 2009). SSR125543 has been reported to produce antidepressant-like effects in several animal models, including the UCMS (Alonso et al., 2004; Griebel et al., 2002b; Louis et al., 2006; Overstreet and Griebel, 2004; Surget et al., 2008b).

2. Experimental procedures

2.1. Animals

Experiments were conducted in male BALB/cByJ mice (Janvier, Le Genest Saint-Isle, France; 8-week-old at the beginning of the experiment). They were maintained under standard laboratory conditions (22 ± 2 °C) and kept on a 12 h light/dark cycle with light onset at 9:00 PM. Food and water were freely available. All procedures have been approved by the Animal Care and Use Committee of Sanofi Research and fully comply with French legislation (decree 87-848, October 19, 1987; and order from April 19, 1988), which implemented the European directive (86/609/EEC) on research involving laboratory animals.

2.2. Drugs

Fluoxetine was provided by Sigma Aldrich (France) and SSR125543 was synthesized by the Medicinal Chemistry Department of Sanofi. They were prepared in saline (fluoxetine) or methylcellulose (0.6%) and Tween 80 (SSR125543). Mice from the stressed control group received saline. Fluoxetine (10 mg/kg) and SSR125543 (20 mg/kg) were administered via intraperitoneal route at a volume of 10 ml/kg. The doses were chosen on the basis of a previous study with these compounds in the UCMS (Surget et al., 2008a).

2.3. Unpredictable chronic mild stress (UCMS)

UCMS-exposed mice were isolated in small individual cages (24 cm × 8.5 cm × 10.5 cm), while non-stressed controls were housed by 5 in standard laboratory cages (42 cm × 28 cm × 18 cm). The protocol consisted of the sequential and unpredictable application of a variety of mild stressors during 8 weeks (Fig. 1). The stressors include altered bedding (change or removal of sawdust, damp sawdust, substitution of sawdust with 21 °C water), 1-h restraint in small plastic tubes, cage tilting (45°), cage exchange (mice were placed in the empty cage of another male), predatory sounds, altered length and time of light/dark cycle.

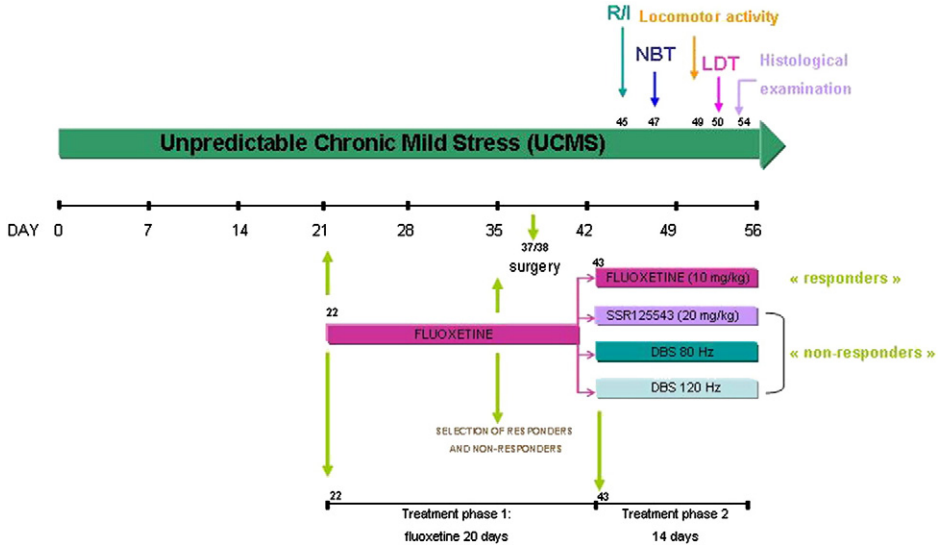

Fig. 1. Experimental design. Three groups of mice were used initially depending on the environment (control/UCMS) and the treatment (vehicle/fluoxetine). From the beginning of week 4 (day 22) until the end of week 6 (day 42), mice received either a saline injection or were treated daily with fluoxetine at 10 mg/kg (Treatment phase 1). At day 35, fluoxetine-treated mice were subdivided into a responder and a non-responder group. These latter were used for bilateral DBS (at two frequencies: 80 or 120 Hz) or were treated with the CRF_1 receptor antagonist, SSR125543 at 20 mg/kg for two weeks from day 43. Responder mice continued to receive fluoxetine until the end of the UCMS, which lasted 8 weeks (Treatment phase 2). Behavioral tests were performed between days 45 and 50. R/I = resident/intruder test; NBT = nest building test; LDT = light/dark test (n = 5 to 14).

The behavioral tests used have been validated by previous studies using the UCMS procedure. The different behaviors assessed in these tests are claimed to correspond to different aspects of depressive symptomatology. According to the DSM-IV-TR classification, a diagnosis of MDD corresponds to the presence of at least five symptoms among nine (American Psychiatric Association, 2000). Therefore, in an attempt to parallel features reminiscent of MDD, we have selected a set of the most representative (and technically addressable) behaviors, which are (1) decreased motivation, (2) psychomotor agitation, and (3) irritability. In addition, as MDD is frequently associated with anxiety disorders, we have assessed anxiety-like behaviors following UCMS exposure. For a comprehensive review of the UCMS procedure and its features, see Surget and Belzung, 2009.

2.4. Selection of fluoxetine-resistant mice

The weight gain and the coat state were assessed weekly. The coat state evaluation involved the assessment of eight different body parts: head, neck, dorsal coat, ventral coat, tail, forepaws, hind paws and genital region. For each body area, a score of 0 was attributed for a coat in good condition or a score of 1 for a dirty and damaged coat. The total score was defined as the sum of the scores for each body part. This index has been pharmacologically validated in previous studies (Griebel et al., 2002a,b; Surget et al., 2008a, 2011). From the beginning of week 4 (day 22) until the end of week 6 (day 42), mice received either a saline injection or were treated daily with fluoxetine at 10 mg/kg. At day 35, fluoxetine-treated mice were subdivided into two groups based on their physical state score: the responders (<2.25) ($n=30$) and the non-responders (≥ 2.25) to fluoxetine ($n=32$). This cutoff value was chosen because the score of coat state of stressed control animals at the end of week 6 averaged 2.25. While responder mice continued to receive a daily administration of fluoxetine at 10 mg/kg, animals from the non-responder group were subsequently used for bilateral DBS [at one of two frequencies: 80 ($n=11$) or 120 ($n=11$) Hz) or were treated with the CRF_1 receptor antagonist, SSR125543 ($n=10$) for two weeks from day 43. We limited the treatment period to two weeks to avoid further degradation of the physical state of vehicle-control animals, which had been submitted to the UCMS regimen for a long period of time (i.e. 8 weeks). Behavioral testing started two days after the beginning of DBS or SSR125543 treatment: third day resident/intruder test, fourth day nest building test, ninth day activity, and tenth day light/dark test. DBS was applied or SSR125543 was administered 1 h prior to testing. Fluoxetine-responder mice were subjected in parallel to the behavioral tests.

2.5. Resident/intruder test

The resident/intruder (R/I) test was modified from previously described protocols (Guillot et al., 1994; Mineur et al., 2003). Control mice were single-housed 24 h before testing. All mice were tested against an 8-week C57BL/6 intruder. The opponent was placed into the home cage of the test animal (resident) so that mice were in opposite corners. Latencies of the first attack were recorded for 6 min. Attacking intruder mice were excluded. Increased aggression in mice subjected to the UCMS has been suggested to relate to aspects of irritability in major depression (Surget and Belzung, 2008a).

2.6. Nest building test

The procedure is based on that described by Deacon (2006). The nesting material consisted of a piece of cotton (2–3 g), which was placed in the homecage. The nests were assessed 24 h later on a rating scale of 1 to 5-point nest-rating scale: 1 = nestlet not noticeably touched, 2 = nestlet partially torn, 3 = nestlet mostly shredded but often not identifiable nest site, 4 = an identifiable but flat nest, 5 = a (near) perfect nest. Reduced nest building has been suggested to model aspects of loss of energy or decreased motivation in major depression (Cryan and Holmes, 2005).

2.7. Activity test

The actimeter device consisted of a cylinder (20 cm diameter, 9.5 cm high, Apelex, France) equipped with two perpendicular light beams located 1.5 cm above the floor. Horizontal locomotor activity was quantified as total number of beams crossed during a 60-min period. Increased locomotor activity following UCMS exposure has been suggested to relate to aspects of psychomotor agitation in major depression (Cryan and Holmes, 2005; Surget and Belzung, 2008b).

2.8. Light/dark test

The test is based on that described by Misslin et al. (1989). The apparatus consisted of two boxes ($20\times20\times14$ cm) covered with Plexiglas. One of these boxes was darkened. A light from a desk lamp, approximately 10 cm above the other box provided the room illumination. An opaque plastic tunnel ($5\times7\times10$ cm) separated the dark box from the illuminated one. At the beginning of the experiment, a mouse was placed in the illuminated box, facing the tunnel. The apparatus was equipped with infrared beams and sensors capable of measuring the following parameters during a 5-min period: (a) time spent in the lit box; (b) number of entries into the lit box (transitions). The results were expressed as an index: mean time spent in the lit box (s) (a) / mean total number of transitions (b). Previous studies have shown that UCMS exposure often leads to changes in anxiety-related behaviors (Surget and Belzung, 2008c).

2.9. Surgery and deep brain stimulation

Animals were anesthetized with a solution of ketamine/xylazine (100/10 mg/kg) injected intraperitoneally and had their heads fixed in a stereotactic instrument (Kopf Instruments) (day 37 or 38). For electrical stimulation, we used polymide insulated stainless platinum electrodes (Plastics One, Roanoke, VA, USA) with diameter of 100 μm. The electrodes were bilaterally implanted into CC (area Cg2) at the following stereotactic coordinates relative to the bregma: anteroposterior +1.24 mm, medial–lateral ±0.4 mm, dorsoventral −1.9 mm (Franklin and Paxinos, 1997). Animals recovered for a period of one week in their homecage.

In all experiments, cingulate electrodes were used as cathodes, and a needle inserted in the neck muscle was used as the anode. Stimulation was conducted in a separate standard cage daily for 1 h with a homemade electrical stimulator at the following parameters: 2.5 V, 90 μs of pulse width, 40 mA intensity, and frequency of either 80 Hz or 120 Hz. These frequencies were selected based on previous studies (Encinas et al., 2011; Hamani et al., 2010a; Toda et al., 2008), which suggested that in mice stimulation above 50 Hz induces neurogenesis, which has been suggested to be a strong correlate of antidepressant treatments (Santarelli et al., 2003). Animals were habituated to the DBS cage and DBS cable for 90 min prior to stimulation.

2.10. Histology and controls

Following completion of the last test, mice from the DBS groups were killed with an overdose of pentobarbital and brains were removed and frozen. Brain slices (50 μm) were subsequently made using a cryostat and stained with cresyl violet. The placement of the microelectrodes was determined for each mouse by an experimenter blind to the behavioral results. Cases where the tip of one or both cannulae was located outside the CC were excluded for statistical analysis (Fig. 2).

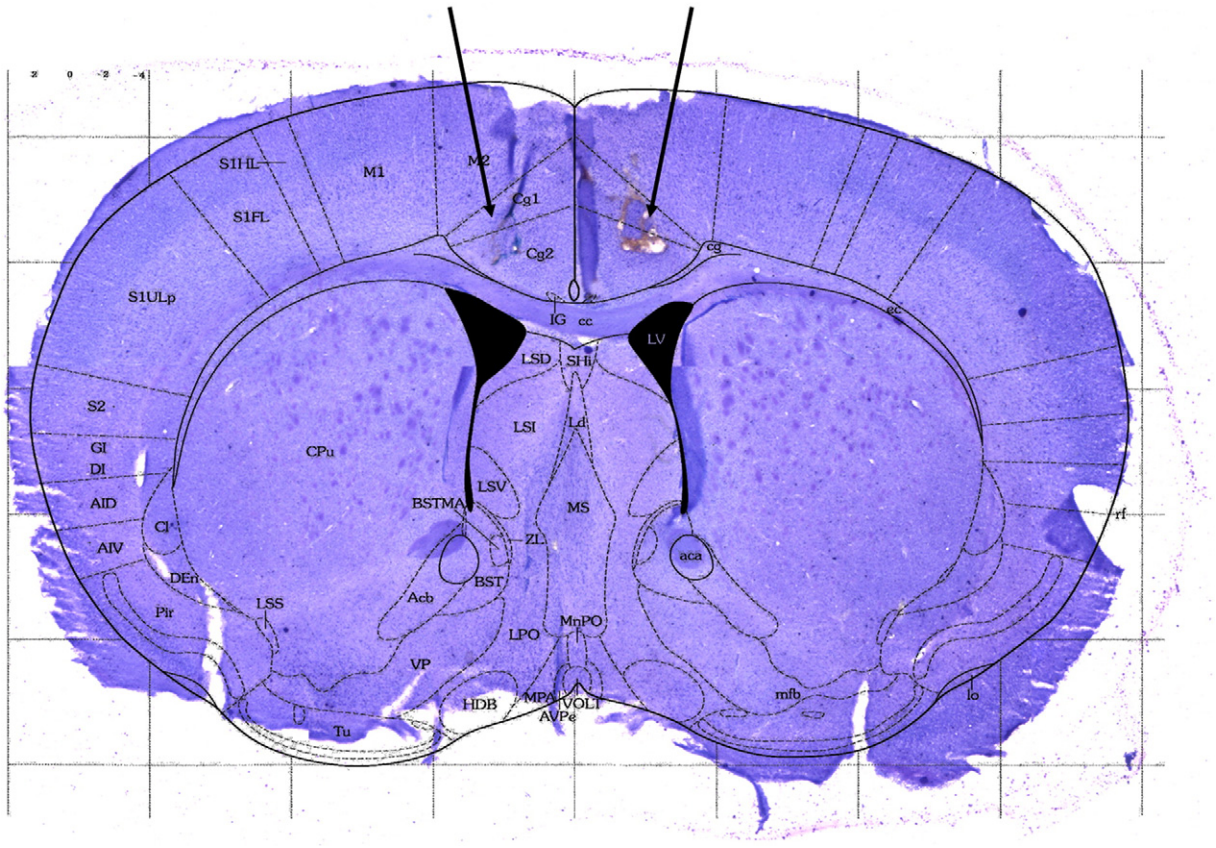

Fig. 2. Position of bilateral stimulation electrodes in the cingulate cortex (area Cg2) on cresyl violet stained brain slices. The figure is adapted from (Franklin and Paxinos, 1997).

2.11. Statistical analyses

Considering that relatively small sample sizes (pre-selection phase: n = 30–32; post-selection phase: n = 4–11) were used and that assumptions for parametric statistics could not be ensured (normality and homoscedasticity using Shapiro–Wilk and Levene tests, respectively), weight and coat state data were rank-transformed and analyzed with a two-way ANOVA (treatment × week) with repeated measures. Significant effects (that is, $P<0.05$) were followed-up with post-hoc tests (Newman–Keuls) when appropriate. Data from the behavioral tests were analyzed using non-parametric Wilcoxon test, Kruskal–Wallis 'analysis of variance by ranks' H-test, or Fisher Exact Probability Test. Significant effects were followed-up with Mann–Whitney post-hoc test with Bonferroni correction when appropriate or Newman–Keuls test. P-values that are indicated in section 3 always derived from the between groups comparisons using the Kruskal–Wallis H-test, whereas P-values resulting from post-hoc comparisons are indicated in the figures.

3. Results

3.1. Body weight, coat state and selection of low fluoxetine responders

3.1.1. Pre-selection phase

Two-way ANOVA with repeated measures showed a significant effect for physical state [$F(10,369) = 50.38$, $P<0.001$] and for weight gain [$F(10,360) = 7.13$, $P<0.001$]. Further analysis indicated that there was a significant degradation of the physical state of the coat of mice due to stress one week after the beginning of UCMS, an effect which lasted in the vehicle-treated group and fluoxetine-treated animals until selection (Fig. 3). Weight gain in fluoxetine-treated animals was significantly smaller in comparison to the other groups at weeks 3, 5 and 6 (data not shown).

3.1.2. Post-selection phase

At the end of week 6 (day 35), mice from the fluoxetine group with physical state scores ≥2.25 (n = 32, i.e. 53% of total fluoxetine-treated mice) were considered as non-responders. Post-selection statistical analysis of the coat state scores ($H = 31.38$, $P<0.001$) showed that mice from this group displayed significantly higher coat scores than vehicle-treated animals, while those from the responder group had significantly lower coat scores when compared to stressed controls (Figs. 3 and 4). Average scores of control mice, non-responders and responders were 2.25, 2.88 and 1.95, respectively. In non-responder mice, fluoxetine was substituted after randomization with DBS at 80 Hz (n = 11), DBS at 120 Hz (n = 11) or SSR125543 (n = 10). Mice that underwent surgery displayed a further degradation of their physical state, probably due to the surgical procedure and post-surgical recovery. Moreover, several mice lost their stimulation cap during the course of UCMS, so that the final number of subjects in the DBS groups was: 6 (DBS 80) and 5 (DBS 120). Two-way ANOVA with repeated measures [$F(5,56) = 4.04$, $P<0.001$] confirmed that in both DBS groups the coat state was significantly degraded as compared to non-stressed animals, but also compared to vehicle-treated mice. While two weeks of SSR125543 treatment were not sufficient to improve significantly the degradation of the physical state in low fluoxetine responder mice, continued treatment with fluoxetine in responder resulted in a significant improvement of coat state at week 7 (day 44) (Fig. 2). Analysis of weight gain did not reveal any significant difference [$F(5,55) = 0.59$, $P = 0.71$].

3.2. Resident/intruder test

Vehicle-treated UCMS mice have been significantly more attacked by the intruder mouse than non-stressed control mice ($\chi^2 = 12.86$, $P<0.001$). This effect of UCMS was observed in mice treated with fluoxetine ($\chi^2 = 9.89$, $P<0.01$) and in the DBS 120 group ($\chi^2 = 5.71$, $P<0.05$),

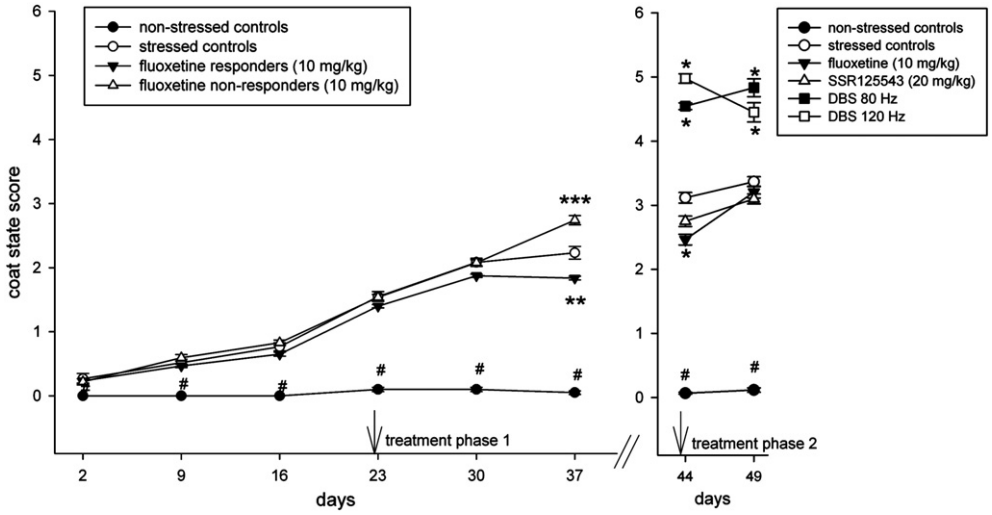

Fig. 3. Physical state alteration during unpredictable chronic mild stress (UCMS) as measured by the coat state of mice. For each body area, a score of 0 was attributed for a coat in good condition or a score of 1 for a dirty and damaged coat. The total score was defined as the sum of the scores for each body part. Effects of repeated treatment with fluoxetine (left and right panels), and repeated treatment with SSR125543 or deep brain stimulation (DBS) in fluoxetine-insensitive mice (right panel). Data represent mean ± sem. # $P<0.05$ (non-stressed vs stressed groups); * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (stressed controls vs drug treatment or DBS).

but not in animals that received SSR125543 and those from the DBS 80 group (data not shown). The latency to first attack of the intruder mouse was significantly shorter in vehicle-treated UCMS animals than in non-stressed mice (Wilcoxon test: $Z=3.25$, $P<0.001$). Statistical analysis of latency scores in all UCMS groups revealed an overall main effect of stress (Kruskal–Wallis: $H=1.9$, $P<0.05$). Post-hoc analysis showed that mice from the DBS 80 group displayed significantly higher latency to first attack than stressed control animals. SSR125543 and DBS 120, but not fluoxetine, showed a clear trend to an increase in this measure, but the effects just failed to reach statistical significance (Fig. 5a).

3.3. Nest building test

Vehicle-treated UCMS mice displayed a significant deficit in nest construction compared to non-stressed animals as indicated by a significant decrease in the nest quality score (Wilcoxon test: $Z=3.74$, $P<0.001$). Statistical analysis of nest scores in UCMS groups did not reveal any significant global effect (Kruskal–Wallis: $H=8.35$, $P>0.05$). However, the effect of SSR125543 just failed to reach statistical significance ($P=0.09$) (Fig. 5b).

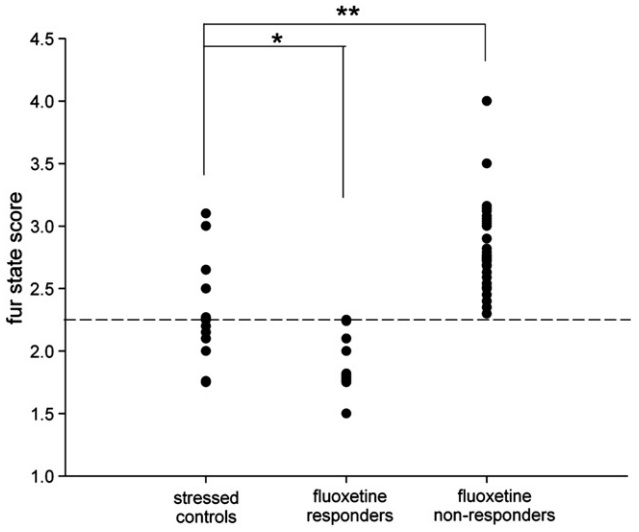

Fig. 4. Distribution of individual coat state scores of stressed controls, fluoxetine responders (<2.25) and fluoxetine non-responders (≥2.25) following 6 weeks of UCMS. * $P<0.05$; ** $P<0.01$ (Newman–Keuls).

3.4. Activity test

Vehicle-treated mice subjected to UCMS displayed a significant increase in horizontal locomotor activity as compared to non-stressed control animals (Wilcoxon: $Z=-3.93$, $P<0.0001$). Statistical analysis of locomotor activity performance in all UCMS groups revealed a significant main effect (Kruskal–Wallis: $H=2.35$, $P<0.05$). Post-hoc analysis showed that DBS at 120 Hz, but none of the other treatments, produced a significant attenuation of this hyperactivity (Fig. 5c).

3.5. Light/dark test

Stressed mice treated with vehicle spent less time for each entry into the lit box than non-stressed animals as shown by a significant decrease in the time/entry index (Wilcoxon test: $Z=3.18$, $P<0.01$). While neither drug treatment modified significantly the index score, DBS at both stimulation intensities increased significantly the time per entry index (Kruskal–Wallis: $H=3.06$, $P<0.01$) (Fig. 5d).

4. Discussion

The present study provides the first evidence that DBS of the CC and repeated treatment with the CRF_1 receptor antagonist, SSR125543, attenuate symptoms reminiscent of aspects of anxiety and depression in a chronic model of depression using mice refractory to the behavioral effects of the antidepressant, fluoxetine.

4.1. The unpredictable mild stress procedure as a model of treatment-resistance

Animal models of treatment-resistant depression are sparse (Samuels et al., 2011). It has been suggested that rodents treated chronically with corticosterone (David et al., 2009) or mutant mice expressing high density of $5\text{-}HT_{1A}$ receptors at the presynaptic level (Richardson-Jones et al., 2011) may represent valid models of antidepressant resistance based on their insensitivity to chronic treatment with fluoxetine in assays measuring different aspects of depressive-like behaviors. Moreover, there have been several reports using variants of the UCMS to model treatment resistance in rodents. In one study, it was demonstrated that if mice are on a

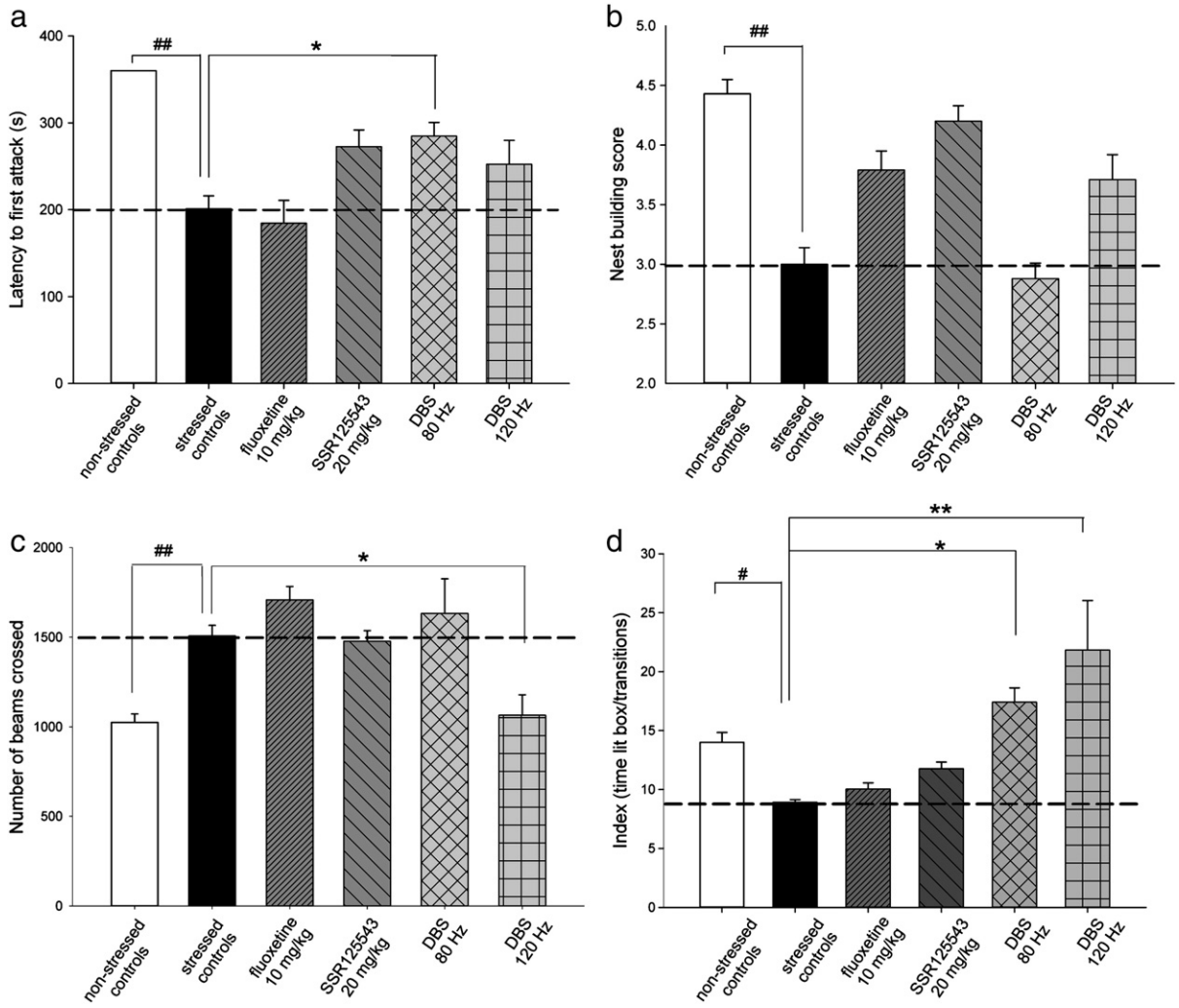

Fig. 5. Effects of repeated administration of fluoxetine (responders), SSR125543 and DBS at 80 and 120 Hz (non-responders) on UCMS-induced anxiety (light/dark test), aggressiveness (resident/intruder test), psychomotor agitation (locomotor activity test) and loss of energy (nest building test). Data represent mean + sem. # $P<0.05$, ## $P<0.01$ (stressed controls vs. non-stressed mice) (Mann–Whitney); * $P<0.05$, ** $P<0.01$ (treated vs. stressed controls) (Newman–Keuls).

high fat diet during multiple UCMS procedures, they become resistant to fluoxetine (Isingrini et al., 2010). Another study in rats showed that chronic treatment with the 5-HT reuptake inhibitor, escitalopram, resulted in a bimodal distribution of the effects of the drug on the main index of depression (i.e., sucrose consumption), where one group recovered (increased sucrose consumption), while another was refractory to treatment (no increase in sucrose consumption) (Jayatissa et al., 2006). A similar approach was employed in the current study but we used the coat state as a measure of depression and antidepressant efficacy to select responders and non-responders. Sucrose consumption, which may be a more parametric measure of depression than the coat state during chronic mild stress has been shown to be rarely sensitive to UCMS when using mice as subjects (Surget and Belzung, 2009). Physical alteration of the coat state has been used extensively in UCMS studies as it has the advantage of permiting the assessment of the evolution of the UCMS-induced effects or the onset of an antidepressant treatment. It was claimed to be the consequence of decreased grooming, an important aspect of the rodent behavioral repertoire, which is very sensitive to stress (for a review, see (Surget and Belzung, 2008d). In line with previous studies using the UCMS, our findings showed that the stress regimen led to a progressive deterioration of the coat state of mice. The fluoxetine-exposed population was found to split into a responder and non-responder group, with a percentage of animals in each group highly comparable.

4.2. Deep brain stimulation of the cingulate cortex in treatment-resistant mice

In the present study chronic intermittent high-frequency (120 Hz) DBS of the CC in treatment-resistant mice improved several behaviors reminiscent of symptoms of major depression such as irritable mood (resident/intruder test), psychomotor agitation (locomotor activity test) and, to a lesser extent, loss of energy (nest building test). Moreover, the complete attenuation of stress-induced increase in anxiety-like behaviors in the light/dark test at both frequencies, suggests that DBS may be useful in anxiety disorders following traumatic stress exposure, such as post-traumatic stress disorder. The 120 Hz stimulation protocol used in this study may have been critical for observing antidepressant-like effects following UCMS. Although there is no consensus (see for example, Andrews, 2010; Pfister and Tass, 2010), brain stimulation of >100 Hz has been reported to be more effective in producing antidepressant effects both in clinical and in rodent models (Hamani et al., 2010a; Mayberg et al., 2005). This may explain the weak efficacy of the 80 Hz stimulation protocol in the current study. Coat state scores could not be exploited in mice subjected to DBS as surgery for the implantation of electrodes produced a further deterioration of the physical state, which makes the measure no longer relevant as an index of depression. It was therefore not possible to assess the onset of DBS

treatment. However, our data showed that DBS of the CC decreased aggressiveness as early as three days after the first stimulation, indicating at least a moderate onset of action. Recent studies demonstrated that DBS of the lateral habenula produces an immediate inhibitory effect on sucrose self-administration or cocaine-seeking behavior, suggesting that DBS has a fast onset of action (Friedman et al., 2010, 2011).

Despite the therapeutic benefits of DBS in TRD and the successful application of this method in rodent model of depression, the mechanism by which stimulation of the CC or SGC alleviates symptoms of depression is unknown. One study found that DBS of the mPFC attenuated partially chronic stress-induced decrease in BDNF, a well-known marker of mood disorders and antidepressant activity (Hamani et al., 2012). The cingulate cortex is part of the mPFC in rats, this latter being the region most commonly suggested as the anatomical correlate of BA 25 of the SGC in this species. More specifically, the homologous of the SGC would be the ventral aspect of the mPFC. The finding that 5-HT depletion abolished these effects suggests that mPFC descending projection neurons targeting subcortical monoaminergic nuclei may be involved. Moreover, a recent study using optogenetic stimulation of the mPFC in socially-defeated mice showed that the antidepressant-like activity of local blue-light application was associated with an activation of excitatory pyramidal neurons and GABAergic interneurons (Covington et al., 2010). Other studies in rodents targeting a different structure have shown that DBS increased plasma and brain tissues concentrations of monoamines (i.e. norepinephrine, dopamine and serotonin) when applied to the habenula, or decreased tyrosine hydroxylase levels while increasing the length of apical and basilar dendrites in pyramidal neurons of the prefrontal cortex, when targeting the nucleus accumbens (Falowski et al., 2011). Whether similar mechanisms account for the antidepressant-like effects observed in the current study using DBS of the CC in the UCMS remain to be determined. A recent study using the UCMS demonstrated changes in gene expression in the CC, which were reversed by chronic treatment with fluoxetine (Surget et al., 2008b). It would be interesting to determine potential transcriptome changes in fluoxetine-resistant mice and, if any, to test if DBS of the CC may be able to reverse them. Finally, it is important to note that a putative generalized model of the effects and mechanism of action of DBS has been proposed by Benabid et al. (2005), which might involve simultaneously or in sequence jamming of neural transmission, direct inhibition of spike initiation at the level of the membrane, decrease release of low molecular weight proteic neurotransmitters and/or retrograde activation of upstream neuronal structures.

4.3. CRF_1 receptor blockade in treatment-resistant mice

Repeated administration of the CRF_1 receptor antagonist, SSR125543, was not as effective as DBS, but it reversed several of the effects of UCMS in fluoxetine-resistant mice. SSR125543 reduced aggressiveness and attenuated, to a lesser extent, the deficit in nest building, but unlike DBS, it failed to modify stress-induced increase in anxiety-like behavior and hyperactivity. It is also worth mentioning that SSR125543 did not significantly attenuate the degradation of the coat state following repeated stress exposure. SSR125543 has been tested several times in the UCMS using a similar stress regimen or variants. In all these studies the drug demonstrated clear antidepressant-like effects at the same (20 mg/kg, i.p.) (Surget et al., 2008a,b, 2011) or lower (10 mg/kg, i.p.) (Griebel et al., 2002b) dosage as used in the current study. However, this is the first time that SSR125543 was tested in mice refractory to the antidepressant, fluoxetine. The idea that CRF_1 antagonists may be useful in the treatment of TRD originates from experiments using the UCMS in mice with focal hippocampal irradiation to disrupt neurogenesis, a hallmark of monoaminergic antidepressant activity (Surget et al., 2008a). They revealed that loss of neurogenesis completely blocked the effects of fluoxetine, but it did not prevent the antidepressant-like effects of SSR125543, suggesting that for CRF_1 receptor antagonists hippocampal neurogenesis is nonessential to exert antidepressant activity, and perhaps might be effective in treatment-refractory depressed patients. The current findings do not convincingly confirm this idea as SSR125543 normalized only a subset of behaviors in treatment-resistant mice. However, it is important to note that the drug was administered only for two weeks, a duration which may have been insufficient to improve significantly depressive-like behaviors in these mice. Our previous studies with SSR125543 showed that the drug produced its peak effect in the UCMS following 3 weeks of treatment (Griebel et al., 2002b; Surget et al., 2008a,b, 2011).

A somewhat unexpected finding of the current study is the lack of effect of fluoxetine in the behavioral tests. The drug significantly improved the degradation of the physical state, but it showed only a non-significant trend in improving nest building, while leaving unaffected the behavioral responses in the other tests. Fluoxetine was tested at a dose which consistently produced antidepressant-like effects in previous UCMS studies, on both coat state and behavioral tests. However, it must be emphasized that the tests used in the former UCMS studies (e.g., splash test, sucrose consumption) were different than those employed in the current study (e.g., nest building, resident/intruder, light/dark). It is possible that the latter, which address somewhat different aspects of depressive-like behaviors in the UCMS (e.g., irritable mood, psychomotor agitation, loss of energy) may have been less sensitive to the action of fluoxetine than those used in former UCMS experiments (e.g., stress coping, anhedonia).

4.4. Methodological considerations

Inherent caveats exist when performing DBS. These caveats include the accuracy of targeting the region of interest. Electrical stimulation is not spatially precise and can cause stimulation, inhibition, or inactivation of surrounding areas. Although we have removed from our analysis mice that did not show optimal placement of the electrode in the CC, it cannot be excluded that the stimulation spread to adjacent regions such as the motor cortex and the corpus callosum, which may have played a role in the current effects. To overcome these limitations, it will be interesting in future studies to use optogenetic, rather than electrical, stimulation, as it allows a precise targeting of regions or cells with high temporal precision (Bernstein and Boyden, 2011; Yizhar et al., 2011). Another important aspect to be discussed is the stimulation period used, which lasted for only 2 weeks. It may not have been long enough to demonstrate all the changes that were occurring. This could partly explain the weak effects in the nest building test. In addition, to mimic more closely the clinical scenario, in which depressed patients receive DBS continuously, current experiments should have been conducted with stimulation being delivered 24 h/day. However, there are major technical difficulties to apply such a protocol of stimulation in mice subjected to repeated, unpredictable stress. The lack of sham group may be another weakness of our study. However, considering that DBS at 80 Hz produced much weaker effects than when applied at 120 Hz, it is unlikely that surgery and electrode placement by themselves may have produced the effects in the behavioral tests.

5. Conclusions

In summary, this study extends recent clinical findings, which demonstrate that DBS within the CC can be effective for treatment-resistant depression. It also suggests that pharmacological blockade of the CRF_1 receptor may provide an additional alternative for these patients. Finally, this study demonstrates that the UCMS may offer the appropriate framework to investigate treatment resistance in preclinical models, with face, construct and predictive validity.

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