# DEEP BRAIN STIMULATION IN TREATMENT-RESISTANT DEPRESSION IN MICE

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## Introduction

- Major depressive disorder (MDD) is a widespread and costly illness, with a prevalence of about 10% worldwide. A variety of treatments is available, but a significant proportion of patients do not achieve sustained symptomatic remission. Indeed, current antidepressants have limited therapeutic efficacy as approximately 30% of patients are treatment-resistant. 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 [1]. It was notably shown that chronic electrical stimulation of white matter tracts adjacent to the subgenual cingulate gyrus resulted in a striking and sustained remission of depression.
- In this study, we used a modified version of the unpredictable chronic mild stress (UCMS) test, a naturalistic model of depression, to validate high-frequency electrical stimulation of the subgenual cingulate cortex (sgCC, i.e. Brodmann area 25) as a possible treatment of drug-resistant depressive-like state.

## Material and Methods

### ANIMALS

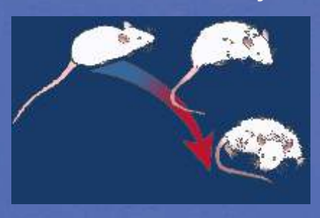
- All experiments were conducted in male BALB/c mice (8-week-old), housed individually, in accordance with the "Guide and Care and Use of Laboratory Animals" (National Institute of Health) and the in-house Animal Ethics Committee.

### UNPREDICTABLE CHRONIC MILD STRESS

- The protocol consists of the sequential and unpredictable application of a variety of mild stressors during 8 weeks. The stressors include altered bedding (change or removal of sawdust, damp sawdust, substitution of sawdust with 21°C water), cage tilting (45°), cage exchange (mice were placed in the empty cage of another male), altered length and time of light/dark cycle etc. For more details, see [2].

### SELECTION OF TREATMENT-RESISTANT MICE

- We evaluated on a weekly basis the physical state of the coat, an index of depressive-like state in these animals. 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. From week 3 until the end of week 5, mice received either a saline injection or were treated with the classical antidepressant fluoxetine. At the end of week 5, fluoxetine-treated mice were subdivided into two groups based on their physical state score: the most responsive (<2.5) to fluoxetine and the less responsive (>2.5) to the drug. The latter were considered as 'treatment-resistant' and were subsequently used for bilateral DBS (at two frequencies 80 or 120 Hz) or were treated with the $CRF_1$ receptor antagonist, SSR125543, based on the idea that the blockade of this receptor may be a possible therapeutic strategy for treatment-resistant patients [3]. Following two weeks of daily 1-h DBS or SSR125543 treatment, mice were tested in a variety of behavioral procedures measuring different aspects of depressive-like behaviors in mice (Figure 1).



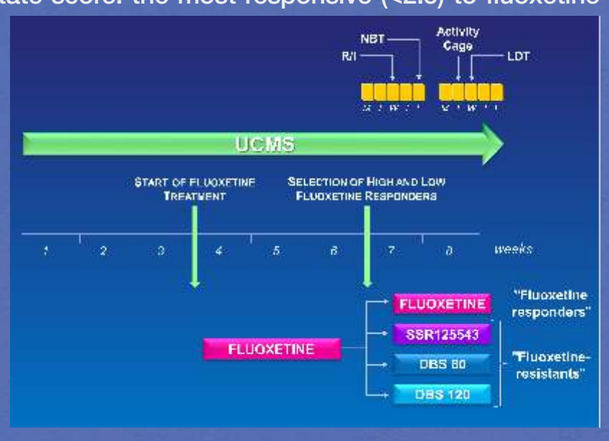
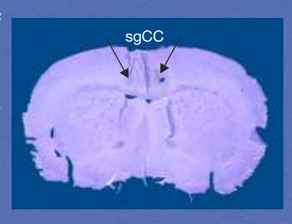



**Figure 1** Experimental design

### DEEP BRAIN STIMULATION

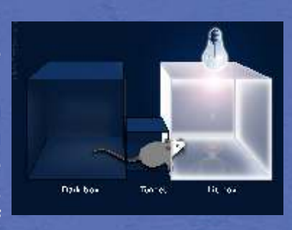
- Stereotaxic surgery was carried out for bilateral implantation of bipolar electrodes in the sgCC. The electrodes were made of two parallel insulated platinumiridium electrode wires, coated with Teflon. Electrodes were inserted bilaterally into the sgCC at the following coordinates in mm relative to bregma: anterior-posterior: +1.24, mediolateral: ±0.4, dorso-ventral: -1.9. Stimulation was bipolar at a frequency of 80 or 120Hz and a pulse width of 30 µsec, 2.5V.



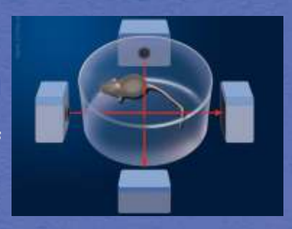

### BEHAVIORAL TESTS

- **Nest Building Test (NBT)**
  A piece of cotton was placed in the home cage allowing the mouse to build a nest. The quality of the nest was evaluated 24 h later using a 5-point nest-rating scale: 1 = cotton not noticeably touched, 2 = partially torn up, 3 = mostly shredded nest, 4 = nest identifiable but flat, 5 = perfect nest or nearly.
- **Resident / Intruder Test (R/I)**
  An unfamiliar male C57BL/6J mouse without particular fighting experience was placed in the home cage of the test mouse. Mice were then allowed to interact freely until the first attack occurred by the resident or until the end of the 6-min experiment. Each intruder mouse was used only once in this test. The latency to first attack was measured. In the absence of attack, a score of 360 sec was given.

- **Light / Dark choice Test (LDT)**
  The apparatus consisted of two boxes 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 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. The results were expressed as an index: mean time spent in the lit box (sec) (a) / mean total number of light box entries (b).



- **Activity Cage**
  Testing was conducted in a small open-field equipped with infrared beams and sensors and placed in sound-attenuated cupboards. Horizontal locomotor activity was quantified as total number of beams crossed during a 60-min period.



### DRUGS

- Drugs were suspended in saline or methylcellulose (0.6%) and Tween 80 (0.1%). Fluoxetine (10 mg/kg) and SSR125543 (20 mg/kg) were administered via intraperitoneal route.

References

[1] Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C (2005). Deep brain stimulation for treatment-resistant depression. Neuron 45: 651-660.

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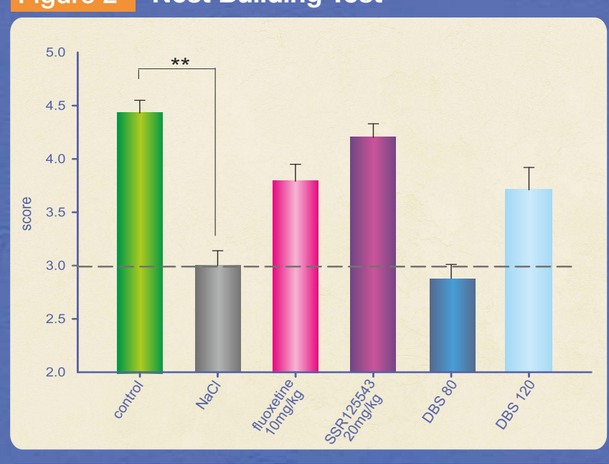
[3] Surget A, Wang Y, Leman S, Ibarguen-Vargas Y, Edgar N, Griebel G, Belzung C, Sibille E (2009). Corticolimbic transcriptome changes are state-dependent and region specific in a rodent model of depression and of antidepressant reversal. Neuropsychopharmacology 34: 1363-1380.

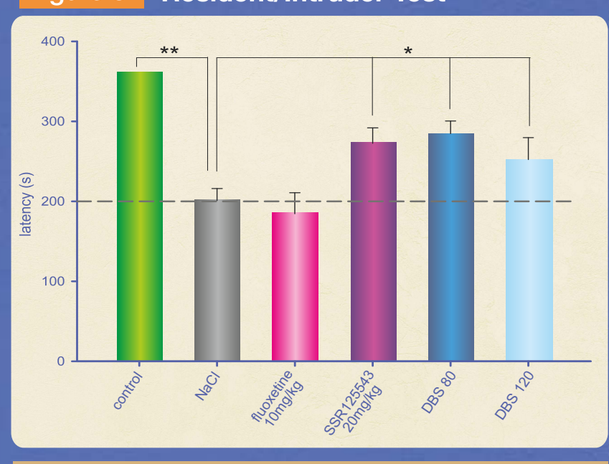
## Results

**Figure 2** Nest Building Test



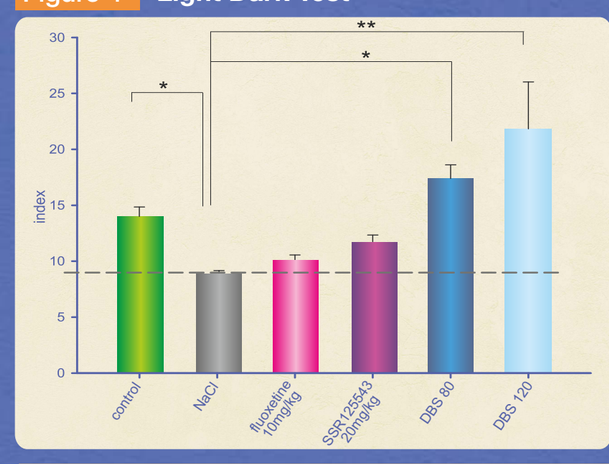

- **UCMS led to an alteration of the efficiency in building a nest.**
- **Repeated treatment with fluoxetine, SSR125543 and DBS at 120 Hz tended to improve the ability of stressed mice to build elaborated nests, albeit these effects just failed to reach statistical significance.**

**Figure 3** Resident/Intruder Test



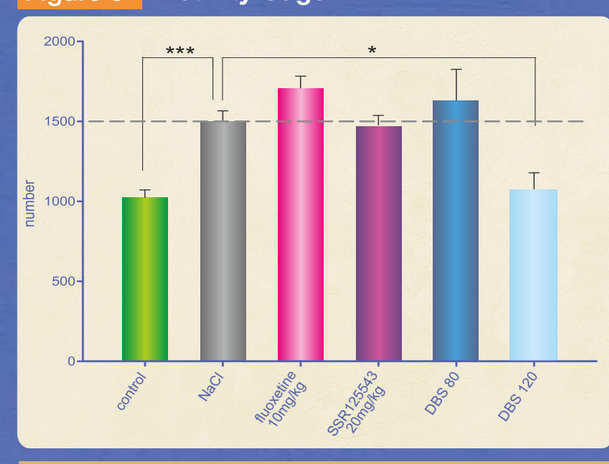

- **UCMS significantly decreased the latency to first attack the intruder.**
- **Repeated treatment with SSR125543 and DBS at both 80 and 120 Hz significantly increased the latency to first attack in stressed mice.**

**Figure 4** Light Dark Test




- **UCMS significantly decreased the average time spent by mice in the lit box for each entry.**
- **Both DBS intensities, but neither of the pharmacological treatment, significantly increased the average time per entry spent by stressed mice into the bright area.**

**Figure 5** Activity Cage




- **UCMS increased significantly spontaneous motor activity of mice exposed to a novel environment.**
- **DBS at 120 Hz, but none of the other treatments significantly attenuated the elevated activity in stressed mice.**

For all the figures, data are mean ± SEM; *p<0.05; **p<0.001; ***p<0.0001 (Wilcoxon or Kruskal-Wallis test).

**Summary table**

| MDD HUMAN | UCMS MOUSE | | | | |
|---|---|---|---|---|---|
| | | | Effects of treatment | | |
| Symptoms | Behavior | Test | SSR125543 | DBS 80 | DBS120 |
| ■ Sadness<br>■ Irritability | ■ Aggression | ■ Resident / Intruder Test | + | + | + |
| ■ Reduced energy | ■ Low motivational mood | ■ Nest Building Test | + | o | + |
| ■ Psychomotor changes | ■ Hyperlocomotion<br>■ Anxiety | ■ Activity cage<br>■ Light Dark Test | o<br>o | o<br>o | +<br>+ |

**+** : improvement of symptoms **O** : no change

## Discussion

- UCMS led to a degradation of the physical state (the result of decreased grooming), increased emotionality and agressiveness, and reduced motivation as shown by the altered efficiency in building a nest. When treatment of fluoxetine was initiated 3 weeks after the beginning of the UCMS, it attenuated the degration of the coat fur, but not all of the animals responded to the drug. These latter were subsequently subjected to DBS or treated by SSR125543.
- Results showed that electrical stimulation of the sgCC at 120 Hz resulted in a normalization of grooming, motivation, anxiety-related behaviors and agressiveness. Similarly, treatment with the $CRF_1$ receptor antagonist modified in a positive manner grooming, motivation and aggressive behaviors, but failed to attenuate anxiety-like responses in 'treament-resistant' mice.

## Conclusion

- **These findings demonstrate for the first time that bilateral DBS of the sgCC and, to a lesser extent, pharmacological blockade of the $CRF_1$ receptor in 'treatment-resistant' chronically stressed 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 patients.**

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No potential conflict of interest