Monoamine oxidase inhibitors potentiate the effects of deep brain stimulation

Supplementary Materials and Methods

All animal protocols have been approved by the Animal Care committee of the Centre for Addiction and Mental Health.

Forced Swim Test (FST)

On the first day of testing, male Sprague-Dawley rats (250-300 g) were individually placed for 15 min in a cylinder (25 cm diameter, 60 cm tall) filled with 25 ± 1 °C water to a depth of 40 cm. On the following day, rats underwent a 5 min swimming session. This was divided into 5 sec segments and the predominant behavior of the animals (immobility, swimming, or climbing) during each segment was blindly scored (maximum of 60; 1 point per segment) (1, 2). In the FST, low immobility is associated with an antidepressant-like effect.

Drug administration

We first conducted dose-response curve experiments with various classes of antidepressants in different groups of rats undergoing the FST. The following medications and doses were tested: fluoxetine (SSRI; 5mk/Kg, 10mg/Kg, 20mg/Kg i.p.); imipramine (tricyclic antidepressant; 3.5mk/Kg, 7.5mg/Kg, 15mg/Kg i.p.); tranylcypromine (MAOi; 1.5mk/Kg, 2.5mg/Kg, 5mg/Kg i.p.); reboxetine (NRi; 7.5mg/Kg, 15mg/Kg i.p.). Drugs were injected 1 and 5 hr after the first swimming session on day 1 and 1h prior to the FST on day 2 (3).

In a second experiment, doses of each medication associated with a 30-40% reduction in immobility scores were given to animals alongside DBS.

Surgical Procedures and Electrical Stimulation

Rats were anesthetized with ketamine/xylazine (100/7.5 mg/kg i.p.) and had electrodes implanted in the vmPFC at the following stereotaxic coordinates: anteroposterior (AP) + 3.0, lateral (L) ± 0.4, and depth (D) 5.6mm (4). Monopolar stainless steel electrodes (250 µm in diameter and 0.75

mm of exposed surface) were used as cathodes. Epidural screws implanted over the somatosensory cortex were used as anodes (AP 0.5, L ± 1.5, D 1.0mm) (4). Controls had holes drilled into the skull but were not implanted with electrodes. Behavioral experiments were conducted seven days after surgical procedures.

Stimulation was conducted with a portable device (ANS model 3510) at 50μA, 130Hz, and 90μsec. This current was **below the threshold to induce a full antidepressant-like response in the FST in our previous studies** (5, 6). As for non-stimulated rats, animals given DBS with the concomitant administration of antidepressant medications were stimulated for 4h on day 1 and 2h prior to the second swimming session on day 2.

Open field test

One week after the FST, animals were given the doses of medications described above combined with DBS and tested in an open field (two doses separated by a 4h interval on day 1 and one dose 1h prior to testing on day 2) (6). Locomotor activity was assessed for 30min in a square 0.49 m^2 apparatus (Med Associates) with infrared photo beams placed along the walls. Crossing of the beams provided counts of motor activity.

Histology

To assess electrode placement, brains were stained with cresyl violet. Electrode location in this study was similar to that published in our previous reports (5-7).

Statistical Analyses

One-way ANOVA followed by Bonferroni/Dunn post-hoc tests were used to compare data when three or more independent groups were considered. Statistical significance was set at $p \leq 0.05$.

Supplementary Results

Dose curve response experiments

Dose curve response experiments were initially conducted to ascertain the dose of different antidepressant medications to be used alongside DBS. Instead of selecting the most effective

doses for each drug, we chose doses associated with a fixed response range (for example 30-40% reduction in immobility scores). This was intended to facilitate comparisons of results across groups receiving different antidepressant medications and DBS in subsequent experiments.

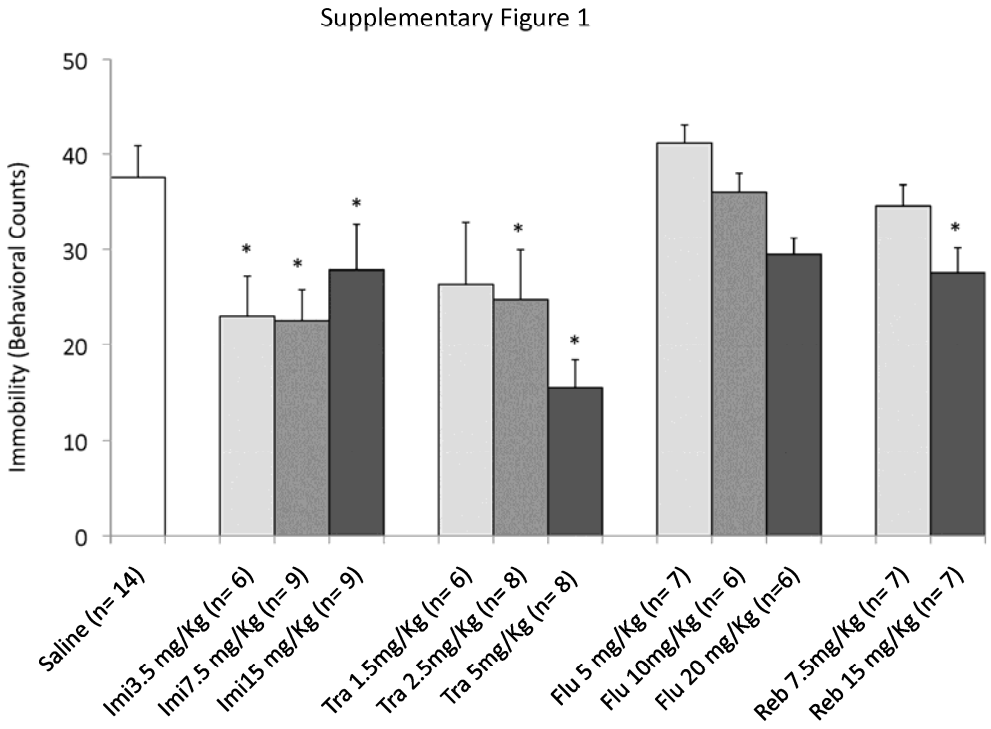
As shown in Supplementary Figure 1, the intended degree of improvement was achieved in animals receiving fluoxetine (20mg/Kg), imipramine (3.5mk/Kg), tranylcypromine (1.5mk/Kg), and reboxetine (15mg/Kg).

Open Field

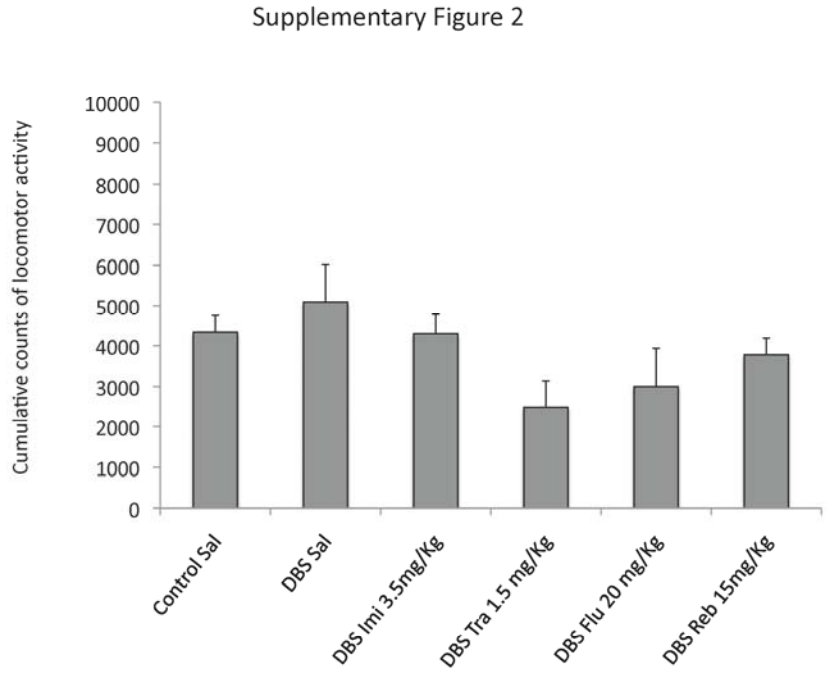
No differences in locomotor activity have been recorded across groups in the open field (Supplementary Figure 2). However, animals receiving DBS and tranylcypromine had a non-significant decrease in locomotion, further indicating that the reduction of immobility observed with this combination of treatments in the FST reflected an antidepressant-like response.

Supplementary Figure Legends

Supplementary Figure 1 Dose curve response experiments carried out to ascertain the dose of different antidepressant medications to be used alongside deep brain stimulation in the forced swim test. Data represent means ± standard error. * statistically significant ($p \leq 0.05$ as compared to controls). Numbers in parenthesis represent animals per group. Imi-imipramine; Tra- tranylcypromine; Flu- fluoxetine; Reb- reboxetine.



Supplementary Figure 2 Assessment of locomotor activity in animals treated with ventromedial prefrontal cortex (vmPFC) deep brain stimulation (DBS) along with different antidepressant medications in the open field. No differences were noticed among groups receiving DBS and saline (Sal), imipramine (Imi), tranylcypromine (Tra), fluoxetine (Flu) and reboxetine (Reb). Data represent means ± standard error.



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