Understanding deep brain stimulation in obsessive compulsive disorder: A preclinical study into the mechanism of action and behaviour
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Deep brain stimulation of the accumbens increases dopamine, serotonin and noradrenaline in the prefrontal cortex

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Abstract

Deep brain stimulation (DBS) of the nucleus accumbens (NAc) is effective in treatment-refractory obsessive compulsive disorder and major depressive disorder. However, little is known about the neurobiological mechanisms underlying the rapid and effective changes of DBS. One of the hypotheses is that DBS modulates activity of monoamine neurotransmitters. In this study we evaluated the effects of DBS in the NAc core on the extracellular concentration of monoaminergic neurotransmitters in the medial (mPFC) and orbital prefrontal cortex (OFC). Freely moving rats were bilaterally stimulated in the NAc core for 2 hours while dopamine, serotonin and noradrenaline using in vivo microdialysis was measured in the mPFC and the OFC. We report rapid increases in the release of dopamine and serotonin to a maximum of 177% and 127% in the mPFC and an increase up to 171% and 166% for dopamine and noradrenaline in the OFC after onset of stimulation in the NAc core. These results provide further evidence for the distal effects of DBS and corroborate previous clinical and preclinical findings of altered neuronal activity in prefrontal areas.
4.1 Introduction

Deep brain stimulation (DBS) is an adjustable and reversible intervention using implanted electrodes to deliver electrical pulses to areas in the brain. DBS of the nucleus accumbens (NAc) demonstrated promising results in treatment-refractory obsessive compulsive disorder (OCD) (Denys et al.2010) and major depressive disorder (MDD) (Malone, Jr. et al.2009; Schlaepfer et al.2008). Furthermore, some case studies reported positive effects of DBS in the NAc on nicotine addiction and alcohol dependency (Mantione et al.2010; Muller et al.2009; Luigjes et al.2011). However, little is known about the neurobiological mechanisms of DBS that may underlie these effects.

Several possible mechanisms have been proposed to explain the effects of DBS in psychiatry (Tye et al.2009). One of the hypotheses is that DBS modulates neuronal network activity and neurotransmitter release (McCracken and Grace2007; Tan et al.2012; Vandehey et al.2009). In view of the pathology of psychiatric disorders such as OCD and MDD, DBS effects on the cortico-striato-thalamo-cortical circuits and the associated modulatory monoamine neurotransmitters are of major interest. It is known that drugs used in the therapy of OCD and MDD elevate the activity of one or more of these monoamines. However, the effects of DBS targeted at the NAc on in vivo neuronal release of monoamines in specific brain areas are currently unknown.

In a previous microdialysis study we found no effect of DBS in the NAc core on local in vivo release of dopamine, serotonin or noradrenaline (van Dijk et al.2011). Recent studies, however, show that DBS in the NAc core may cause region-specific alterations in local field potential and neuronal responses in both the medial and orbital prefrontal cortex (mPFC and OFC) (McCracken and Grace2007; McCracken and Grace2009). The mPFC and the OFC are areas of interest since imaging studies have shown abnormal metabolic activity in both areas in OCD and MDD patients, which normalized following successful pharmacological treatment (Deckersbach et al.2006; Graybiel and Rauch2000; Evans et al.2006).

The objective of this present study is to extend our previous finding by evaluating the effects of DBS in the NAc core on the extracellular concentration of monoaminergic neurotransmitters in the mPFC and OFC. We measured dopamine, serotonin, and noradrenaline using in vivo microdialysis in the mPFC or the OFC one hour before and during stimulation in the NAc core.

4.2 Material and Methods

The experimental procedures are extensively described by van Dijk et al (van Dijk et al.2011). In short, we stereotaxically implanted a microdialysis probe in either the mPFC (12° in the
coronal plane, A + 3.5 mm, L ± 1.8 mm, V -5.5 mm) or the OFC (12° in the sagittal plane A + 4.2 mm, L ±2.5 mm, V -5.8 mm) in combination with two electrodes bilaterally into the NAc core (A +1.3mm, L ±2.0mm, V -8.0mm) in male Wistar rats (250-350g, Harlan, Boxmeer, the Netherlands). The microdialysis probe had an exposed membrane of 2 mm in the OFC and 3 mm in the mPFC (Feenstra and Botterblom 1996). After a recovery period of 7 days microdialysis experiments were performed during the dark cycle (Feenstra et al.2000). Experiments were carried out on 2 consecutive days in a separate room with an experimental setup for 2 rats. Microdialysis samples were collected for 3 hours, every 15 minutes on two consecutive days in vials containing 7.5 µL acetic acid. After 1 hour of baseline recording, a rat was either sham stimulated or stimulated with 300µA (constant current, 120Hz, biphasic, pulse width 80 µs) in random order for 1 hour and 45 minutes each day. The lag time of the system was not corrected for. The first sample that is indicated in the figures after the start of the stimulation contains approximately 7 minutes of ‘unstimulated’ sample. The behaviour of the animals was videotaped and closely observed during the onset and offset of the stimulation. This procedure was repeated on day 2.

All microdialysis samples were stored at -80°C until HPLC analysis. Dopamine, noradrenaline and serotonin were analyzed using a HPLC ALEXYS 100 2D system equipped with electrochemical detection (DECADE II) from ANTEC Leyden. (Zoeterwoude, the Netherlands). The detection limits in a 5 µl sample (signal to noise ratio = 3) varied between 0.01 and 0.02 nM for the monoamines.

After completion of the experiment, all rats were given an electrolytic lesion of 100 µA direct current for 20 seconds to allow subsequent localization of the electrode tip. Thirty µm coronal sections were stained with Cresyl violet for determination of the exact location of the two electrodes and the microdialysis probe.

Baseline values of extracellular monoamine concentrations were defined as the mean of the last four samples before stimulation. All data were then converted to a percentage of the baseline (set as a 100%). All data are presented as mean ± S.E.M. and statistically analysed using two way Repeated Measures Analysis of Variance (RM-ANOVA). We used a between subject design for statistical analysis because due to practical problems (described in 3.1) data from one of the two experimental days was excluded in several animals. The effect of DBS was assessed by using stimulation as the ‘between subjects’ factor and time as ‘within subjects’. If indicated by the Mauchly’s test of sphericity, the number of degrees of freedom where adjusted by a Greenhouse-Geisser correction. If appropriate this was followed by a test of within-subjects contrasts (simple) analysis in which all experimental samples (sample 2 till 11) are compared to the first baseline sample (sample 1).
4.3 Results

4.3.1 Inclusions

Animals were included in the data analysis when histological examination showed correct placement of the electrodes in the NAc core and the probe in the mPFC or the OFC (fig. 1). If one or both of the electrodes was placed incorrectly only the data obtained during the sham stimulation experiment was included. Further exclusion of animals or microdialysis data was done after problems with HPLC-analysis such as incorrect separation of the monoamines or concentrations below detection limit or practical problems such as blockage of the microdialysis probe. In the mPFC group 28 rats were operated of which, after histological and microdialysis examination, 16 animals were (partially) analyzed. This led to 14 sham stimulated rats and 10 rats stimulated with 300 µA in the mPFC group. In the OFC group 21 rats were operated of which 16 animals were (partially) analyzed. This led to an OFC group consisting of 12 sham stimulated rats and 5 stimulated rats for dopamine, 14 sham stimulated rats and 6 stimulated rats for serotonin and 11 sham stimulated rats and 4 stimulated rats for noradrenaline.

Fig 1: Localization of the electrodes in the NAc core and the microdialysis probes in the mPFC and the OFC of the included animals

4.3.2 Behavioural observations

No reproducible alterations in behaviour were observed when the stimulation was switched on. In addition, no abnormal behaviour of the animals was observed during the time that stimulation was on.
4.3.3 mPFC

4.3.3.1 Dopamine

Dopamine in the mPFC was significantly increased by DBS in the NAc core to a maximum of 177% compared to baseline (time×stimulation: F = 3.214, p = 0.025; between- subjects: F = 8.010, p = 0.01). Figure 2 shows the relative change of dopamine level after the start of stimulation. Tests for within-subjects contrasts resulted in a trend or a significant time×stimulation effect for dopamine on time point 5 (F = 5.707, p = 0.026), 6 (F = 3.178, p = 0.088) and 7 (F = 6.360, p = 0.019) compared to time point 1. The average basal level in the mPFC for dopamine was 0.180 nM.

4.3.3.2 Serotonin

DBS in the NAc core increased serotonin levels in the mPFC to a maximum of 127% compared to baseline. An ANOVA with repeated measures showed a significant effect of stimulation (F = 15.017, p = 0.001) and of the time×stimulation interaction (F = 3.403, p = 0.008) for serotonin. This effect was evident at time point 7 (F = 6.362, p = 0.019) and 8 (F = 3.115, p = 0.091). Figure 2 shows the relative change of serotonin compared to four baseline samples after start of stimulation. The average basal level of serotonin in the mPFC was 0.366 nM.

4.3.3.3 Noradrenaline

Statistical analysis did not reveal a significant effect of stimulation on noradrenaline in the mPFC. Figure 2 shows the relative change of noradrenaline compared to four baseline samples after start of stimulation. The average basal level of noradrenaline in the mPFC was 0.203 nM.

4.3.4 OFC

4.3.4.1 Dopamine

DBS in the NAc core significantly increased dopamine levels in the OFC (time×stimulation: F = 2.793, p = 0.025; between- subjects: F = 6.269, p = 0.024). This led to a maximum increase of 171% compared to baseline at time point 6 (F = 8.692, p = 0.01). The average basal levels in the OFC for dopamine respectively were 0.073 nM. Figure 2 shows the relative change of dopamine level after the start of stimulation.

4.3.4.2 Serotonin

In the OFC, DBS in the NAc core had no effect on serotonin levels. Figure 2 shows the relative change of serotonin compared to four baseline samples after start of stimulation. The average basal level of serotonin in the OFC was 0.30 nM.
Deep brain stimulation of the accumbens increases dopamine, serotonin and noradrenaline in the prefrontal cortex.

Fig 2: Effect of DBS in the NAc core on release of dopamine, serotonin and noradrenaline in the mPFC and the OFC. Extracellular levels are expressed as a percentage (means ± S.E.M.) of a baseline of each animal. Rectangular: duration of the DBS, square: sham stimulated group, diamond: 300 µA stimulated group.
4.3.4.3 Noradrenaline

Noradrenaline levels in the OFC significantly increased to a maximum of 166% compared to baseline during DBS in the NAc core (time×stimulation effect: F = 4.294, p = 0.002; between-subjects: F = 14.657, p = 0.002). This increase of noradrenaline was evident at time point 5 (F = 5.885, p = 0.031), 6 (F = 27.038, p < 0.0001), 7 (F = 7.531, p = 0.017), 8 (F = 8.190, p = 0.013), 9 (F = 8.768, p = 0.011) and 10 (F = 5.269, p = 0.039) compared to time point 1. The average basal level of noradrenaline in the OFC was 0.182 nM. Figure 2 shows the relative change of noradrenaline compared to four baseline samples after start of stimulation.

4.5 Discussion

The present study is the first to provide direct evidence that DBS in the NAc core increases in vivo monoamine release in prefrontal areas in freely moving animals. We report rapid increases in the release of dopamine and serotonin in the mPFC, and dopamine and noradrenaline in the OFC after onset of stimulation in the NAc core. In an earlier study we found no effect of DBS in the NAc core on local in vivo monoamine release (van Dijk et al.2011). These results provide further evidence for the distal effects of DBS and corroborate previous clinical and preclinical findings of altered neuronal activity in prefrontal areas (McCracken and Grace2007; McCracken and Grace2009; Rauch et al.2006).

To date, the effect of electrical stimulation of the NAc (van Kuyck et al.2007) on monoamines received little attention. Two studies reported on the effect of NAc stimulation on mPFC monoamine and metabolite postmortem tissue levels. Sesia et al observed no alterations after acute stimulation, while Falowski et al demonstrated a decrease of catecholamines concentrations after chronic, continuous stimulation (Falowski et al.2011; Sesia et al.2010). Our study is the first to provide a measure of in vivo alterations of neurotransmitter activity in the PFC following NAc stimulation in awake animals. There are no direct projections from the NAc core to the mPFC or the OFC as opposed to the direct anatomical projections from both areas to the NAc core (Graybiel and Rauch2000; Haber et al.1995; Voorn et al.2004). The question therefore is: How does DBS in the NAc core modifies neurotransmitter release in the PFC? The common factor underlying the clinical effect may be the stimulation of corticofugal tracts, i.e. white matter (Lehman et al.2011). While NAc proper consists for the most part out of grey matter, there are white matter fascicules passing through, probably representing corticofugal fiber bundles, as described for primates (Lehman et al.2011). We hypothesize that increases in the monoamine release in the PFC could result from antidromic activation of these corticofugal fibers, either targeting striatal neurons or passing through the accumbens as fiber bundles. Indeed, stimulation of the NAC has been shown to change prefrontal neuronal activity through antidromic activation of corticostratial fibers in rats (McCracken and Grace2007).
Antidromic stimulation of corticofugal fibers has also been described for stimulation of other common targets for DBS, such as the subthalamic nucleus (Devergnas and Wichmann 2011). Alternatively, stimulation of accumbens efferents or passing fibers could modulate activity of the cortico-striato-thalamo-cortical loop or the monoaminergic neurons in the midbrain and the pons which project to the PFC. Direct stimulation of monoaminergic fibers from the midbrain and brainstem projecting to the PFC is unlikely as e.g. the dopaminergic fibers are reported to run outside the borders of the NAc (Björklund and Lindvall 1984). Moreover, dopaminergic fibers are non-myelinated and are therefore more difficult to stimulate than the myelinated fibers that are often suggested to be the primary target of DBS (Kringelbach et al. 2007).

Interestingly, the rapid increase in prefrontal monoamine release that we report bears similarity to the effect of combined SSRI and antipsychotic therapy, often used in treatment resistant OCD and MDD (Denys et al., 2004a). This combination also lead to a simultaneous increase of monoamines in both the mPFC and the OFC (Denys et al. 2004; Huang et al. 2006), although it should be noted that the drug-induced increases in 5-HT release are clearly higher. The similarity in these direct effects may indicate a possible common factor of these effective therapies for OCD and MDD.

A direct comparison of the effects of stimulation between the mPFC and OFC suggests that monoamine increases in the mPFC are more enduring than in the OFC. Literature on in vivo monoamine release in OFC is sparse and cannot provide an explanation of the differential effects between the medial and orbital cortex. The mPFC projects mainly to the medial part of the NAc core while the OFC projects more to the central-lateral part of the NAc core (Voorn et al. 2004). However, the electrode positions in the accumbens are such that both medial and orbital PFC afferents may have been activated (Fig. 2).

There are some limitations to our study. First, in our previous study we found no effect on local monoamine release in the NAc core with unilateral stimulation. It is possible that bilateral stimulation has a different effect on monoamine release locally or in distal areas than unilateral stimulation. On the other hand, several clinical and animal studies reported similar effects of unilateral and bilateral stimulation, suggesting that they are equal (Hamani et al. 2010; Hershey et al. 2008; Huff et al. 2010). Second, we stimulated only for a short period of time and the above-mentioned difference between acute and chronic stimulation on tissue levels suggests that long term stimulation might result in different effects. Future studies, using stimulation periods of at least a couple of days should provide a better comparison with the effects of drug treatments and with clinical chronic stimulation.
In conclusion, our study shows that DBS in the NAc core has a rapid effect on the monoamine release in the mPFC as well as the OFC. This suggests that activation of monoamine release in the prefrontal areas may be involved in the early effects of DBS in the NAc following the stimulation. These results in combination with earlier findings of lack of changes of local monoamine release following DBS in the NAc core underline the importance of the effects of DBS in connected areas. Though DBS is used in clinical settings, the lack of knowledge of the mechanism of action of DBS prevents the full potential of its application. DBS has been proposed to modulate circuit activity in general and to normalize pathological deviations of normal circuit oscillations and connectivity (McCracken and Grace 2009; McIntyre et al. 2004). Our finding of a prefrontal increase in monoamine release could be viewed as one of the building blocks to achieve this.
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