Understanding deep brain stimulation in obsessive compulsive disorder: A preclinical study into the mechanism of action and behaviour
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Chapter 3

Unilateral deep brain stimulation in the nucleus accumbens core does not affect local monoamine release

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Abstract

Recent publications have shown promising results of deep brain stimulation (DBS) in the nucleus accumbens for patients with obsessive compulsive disorder and major depressive disorder. Despite its increasing application in the clinical setting, the neurobiological mechanism of action of DBS is still uncertain. One of the possible effects of DBS might be phasic or tonic changes in monoamine release either locally in the target area or in a distant, connected region.

In the present study we investigate whether unilateral DBS of the Nucleus Accumbens Core (NAc core) has a local effect on in vivo monoamine release. Freely moving animals were unilaterally stimulated with 300µA or 400µA (120Hz, pulse width 80 µs) in the NAc core for 5 hours. One hour before and during stimulation we measured dopamine, serotonin, their metabolites and noradrenaline using in vivo microdialysis. We found no significant effect of stimulation on extracellular concentrations of monoaminergic neurotransmitters or their metabolites in the NAc core during stimulation. Our results suggest that the rapid effects of DBS in the NAc are not a result of changes in local monoamine release in the NAc core. For future directions it is interesting to note that several microdialysis and electrophysiology studies have shown effects of DBS in areas distant from the stimulation target.
3.1 Introduction

Recently, deep brain stimulation (DBS) of the nucleus accumbens (NAc) has demonstrated promising results in several psychiatric diseases. Clinical trials have shown that DBS of the NAc may be an effective and safe treatment for treatment-refractory obsessive compulsive disorder (OCD) (Denys et al. 2010) and major depressive disorder (Malone, Jr. et al. 2009; Schlaepfer et al. 2008). Some case studies have shown positive effects of DBS in the NAc on alcohol dependency and nicotine addiction (Mantione et al. 2010; Muller et al. 2009).

DBS is a new technique, which involves the implantation of one or two electrodes into the brain to allow electrical manipulation of specific brain regions. Interest in DBS has increased due to the reversible nature of the technique, the relative safety of the surgery involved and its effectiveness in therapy-resistant patients in neurology as well as in psychiatry. Furthermore, the ability to adjust the stimulation parameters allows for individual tuning to achieve optimal results. Despite its efficacy in the clinical setting, the mechanism of action of DBS is still uncertain. One of the first hypotheses assumed that DBS causes a functional lesion by suppressing neural activity at the stimulated brain area. This suppression may be achieved by a depolarization blockage, synaptic inhibition or synaptic depression (McIntyre et al. 2004). However, there is also evidence that DBS may activate axons which results in a wide range of effects on local cells and on system levels. These effects involve synaptic inhibition, excitation and prodromic or antidromic activation (Deniau et al. 2010). They may then alter the efflux of neurotransmitters, neuropeptides and retrograde messengers (Tye et al. 2009) or the synchronisation of neural circuits (McCracken and Grace 2009). The latter effect is in line with the hypothesis that DBS results in a modulation of the pathological activity in the neuronal network of the stimulated brain area (McIntyre et al. 2004).

The NAc is part of the ventral striatum and is one of the main components of the basal ganglia. The NAc receives input from the amygdala, hippocampus and the prefrontal cortex (PFC) (Nicola 2007). Monoaminergic afferents gating these inputs and modulating accumbens activity are mainly dopaminergic, originating in the ventral tegmental area, medial substantia nigra and the retrorubral cell groups, but also serotonergic from the dorsal and median raphe nuclei and noradrenergic from the locus coeruleus (Groenewegen et al. 1996).

Histological and functional evidence suggests that the NAc can be divided into two subregions. The medial, ventral and lateral portion of the NAc are considered to be the shell of the NAc (NAc shell) whereas the central and dorsal portions are commonly referred to as the core of the NAc (NAc core) (Groenewegen et al. 1999). The NAc core projects preferentially to classical striatal targets such as the pallidal and nigral complex. In contrast the NAc shell connects with striatal output areas such as the lateral hypothalamic areas, dopaminergic cell groups and...
caudal mesencephalic areas that have been associated with locomotor functions (Voorn et al.2004).

The aim of the current study is to evaluate the effect of unilateral DBS in the NAc core on the extracellular concentration of monoaminergic neurotransmitters in the target region. An important indication that stimulation might be expected to affect local monoamine release comes from in vitro studies showing an increase in dopamine release in the NAc after stimulation of the same area (Davidson and Stamford1993;Trout and Kruk1992). Furthermore, taking into account that SSRIs, the first choice treatment for OCD as well as depression, manipulates serotonin levels and that there is a possible hyperdopaminergic state in the basal ganglia for OCD patients (Denys et al.2004), our hypothesis is that DBS may alter monoaminergic activity.

3.2 Material and Methods

3.2.1 Subjects

Male Wistar rats (250-350g, Harlan, Boxmeer, the Netherlands) were housed socially (2-4 animals per cage) in a temperature- and humidity controlled vivarium with a 12-h light/dark cycle (lights on 7:00, off 19:00) and were provided with food and water ad libitum. Rats were handled regularly for 7 days prior to surgery. The study was conducted in accordance with governmental guidelines for care of laboratory animals and approved by the Animal Experimentation Committee of the Academic Medical Centre Amsterdam, the Netherlands.

3.2.2 Surgery

On the day of surgery the rats were anesthetized with intramuscular Hypnorm (0.22mg/kg fentanyl citrate and 7mg/kg fluanisone, VetaPharma) and subcutaneous Dormicum (1.5mg/kg midazolam, Roche) was given for muscle relaxation. The animals were then placed in a Kopf

![Fig 1: A picture of the custom-made electrode/probe combination](image)
stereotactic apparatus (Kopf Instruments, Tujunga, CA) and kept at a constant temperature of 36.5°C using a rectal thermometer connected to a heating pad. A small incision was made to expose the skull after which lidocaine was used for local anaesthesia. A bipolar electrode was stereotaxically implanted together with a custom-made concentric microdialysis probe (Feenstra and Botterblom1996) into the NAc core (A +1.3mm, L ±2.0mm, V -8.0mm). The coordinates are relative to bregma according to Paxinos and Watson, 2007. The bipolar electrode consisted of two twisted teflon coated, platinum/ iridium wires with a diameter of 60 µm and with one pole 500 µm ventral to the other to cover a substantial area of the NAc core. The tips of the wires were cut off straight which formed an exposed surface area per pole of 2.83∙10⁻³ mm². The microdialysis probe had an exposed membrane of 1 mm. The electrode was attached 0.5 mm away from the microdialysis probe with epoxy-glue. The electrode/ microdialysis probe combination was stereotaxically implanted with the electrode medial to the probe. The electrode and probe were fixed to the skull surface with 3 stainless steel screws and dental acrylic cement. Rats were given subcutaneous Temgesic (0.03 mg/kg buprenorfine hydrochloride) post-operatively for pain management and were then housed in individual cages.

3.2.3 Experiments

After a recovery period of 7 days microdialysis experiments were performed during the light cycle (Feenstra et al.2000). Experiments were carried out on 4 consecutive days in a separate room with an experimental setup for 4 rats. At the end of day 1 rats were transferred to the room and connected with PEEK (polyetheretherketone)-tubing (0.51mm outside diameter, 0.013mm inside diameter; Aurora-Borealis, Schoonebeek, the Netherlands) to a dual channel swivel (Instech Lab. Inc, Plymouth Meeting, PA) in a fluid swivel mount on a 4 channel commutator (Plastics One Inc., Roanoke, VA, USA). The dual channel swivel was connected to microinfusion syringes (2.5 ml, 10.92 mm outside diameter, 7.20 mm inside diameter, KLOEHN Inc., Las Vegas, NV, USA), that were placed in a syringe pump (KDS 220, KD Scientific, Zoeterwoude, the Netherlands). Probes were then perfused with Ringer solution (147 mM NaCl, 2.3 mM KCl, 2.3 mM CaCl₂ 1 mM MgCl₂, pH 5.5-6) at a constant flow rate of 1µL/min. Perfusion was kept on overnight and samples were collected for 6 hours, every 30 minutes on three consecutive days in vials containing 10µL acetic acid. After 1 hour of baseline recording, a rat was either sham stimulated, stimulated with 300µA or 400µA (constant current, 120Hz, biphasic, pulse width 80 µs) in random order for 5 hours each day. Stimulation parameters of 300µA or 400µA and a pulsewidth of 80 µs resulted in respectively a charge per phase (Q) of 0.024 µC/phase and 0.032 µC/phase and a charge density per phase (D) of 849 µC/ cm²/phase and 1131 µC/ cm²/phase. This results in a k of 1.31 and 1.56 (Log (D) = k-log(Q)) which is in the safe range where no tissue damage is observed (McCreery et al.1990;Shannon1992). Stimulation of the rats was performed using an isolated stimulator (DS8000, World Precision...
Instruments, Sarasota, FL, USA) and an isolator (DLS100, World Precision Instruments, Sarasota, FL, USA). Sham stimulated animals were stereotactically implanted with a bipolar electrode and a custom-made concentric microdialysis probe in the NAc core and connected to the experimental setup but no electrical stimulation was applied. The behaviour of the animals was closely observed at 30 min intervals and during the onset and offset of the stimulation. All samples were stored at -80°C until HPLC analysis. Dopamine, noradrenaline and serotonin and the metabolites of dopamine and serotonin were analyzed using a HPLC ALEXYS 100 2D system equipped with electrochemical detection (DECADE II) from ANTEC Leyden. (Zoeterwoude, the Netherlands) Samples of 14 µl were spliced in 2 parts of 5 µl and injected on an ALF-105 (50x1 mm, 3 µm C18) column to analyze dopamine and serotonin and on an ALF-115 (150x1 mm, 3µm C18) column to analyze noradrenaline, DOPAC, 5-HIAA and HVA. The mobile phase for the ALF-105 column consisted of 50 mM phosphoric acid, 8 mM KCl, 2.15 mM octanesulphonic acid (OSA) and 12.5 % methanol in milliQ water adjusted to a pH of 6.0. The mobile phase for the ALF-115 column consisted of 50 mM phosphoric acid, 50 mM citric acid, 8 mM KCl, 0.1 mM EDTA, 2.15 mM OSA and 10 % methanol in milliQ water adjusted to a pH of 3.25. The flow rate was kept constant at 0.05 µl/min. Separation was performed at 35 °C and the electrochemical potentials were set at 350 mV for the ALF-105 column and 450 mV for the ALF-115 column against an Ag/AgCl reference in the ISAAC electrochemical cell. The signals were analyzed using Clarity (2.6.4.402) software. The detection limits in a 5 µl sample (signal to noise ratio = 3) were 0.02 nM for the monoamines and 0.2 nM for the metabolites.

3.2.4 Histological examination

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 and were injected with pentobarbital. After the injection, animals were perfused intracardially with 0.9% NaCl followed by 4% buffered formalin. The brains were then removed and placed in 4% buffered formalin for at least 48 hours, followed by 30% sucrose solution until the brains were saturated. Coronal sections of 30µm were cut on a cryostat and stained with Thionin Blue for determination of the exact location of the electrode and microdialysis probe.

3.2.5 Data analysis

Baseline values of extracellular monoamine concentrations were defined as the mean of the last two samples before stimulation. All data where 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). The effect of DBS was assessed by using stimulation as the ‘between subjects’ factor and time as ‘within subjects’. The criterion for significance was set at p < 0.05.
3.3 Results

3.3.1 Histology

Animals were included in the data analysis when histological examination showed correct placement of the electrode/probe combination in the NAc core (fig 2). The sham stimulated and the 300 µA group consisted of 6 rats whereas the 400 µA group consisted of 5 rats.

![Figure 2: Localization of the electrode/probe combination of the included animals.](image)

3.3.2 Behavioural observations

No abnormal behaviour of the animals was observed during the time that stimulation was on. The most frequently observed behaviour was sleeping which concurs with the fact that the stimulation was on during the light cycle.

3.3.3 Dopamine, DOPAC and HVA

Figure 3A shows the relative change of dopamine level after the start of stimulation. Two-way RM-ANOVA revealed a significant effect of time for dopamine (F = 4.925, p < 0.0001), DOPAC (F = 1.875, p = 0.0466) and HVA (F = 7.519, p < 0.0001). However there were no significant effects of stimulation or of the interaction for either three which shows that stimulation has no effect on the release of dopamine, or the formation of DOPAC and HVA. The average basal levels in the NAc core for dopamine was 4.20 nM, DOPAC 142 nM and HVA 302 nM.

3.3.4 Serotonin and 5-HIAA

Two-way RM-ANOVA did not reveal significant changes in stimulation, time and interaction for serotonin. There was a significant effect on time for 5-HIAA (F = 4.038, p < 0.0001) but not on stimulation and interaction. This suggests that stimulation had no effect on the release of...
Fig 3: Effect of DBS in the NAc core on: (A) dopamine, (B) serotonin and (C) noradrenaline in the NAc core. Extracellular levels are expressed as a percentage (means ± S.E.M.) of a baseline of each animal. Rectangular: duration of the DBS, circle: sham stimulated group, square: 300 µA stimulated group, triangles: 400 µA stimulated group.
serotonin or the formation of 5-HIAA. Figure 3B shows the relative change of serotonin compared to two baseline samples after start of stimulation. The average basal levels of serotonin in the NAc core was 0.45 nM and for 5-HIAA 161 nM.

3.3.5 Noradrenaline

Figure 3C shows the relative change of noradrenaline compared to two baseline samples after start of stimulation. Two-way RM-ANOVA did not reveal significant changes in stimulation, time and interaction. The average basal level of noradrenaline in the NAc core was 0.39 nM.

3.4 Discussion

This article reports about measurements of monoamine release by in vivo microdialysis during DBS of the NAc core at the stimulation site. Using freely moving animals we found that unilateral DBS of the NAc core with 300µA or 400µA (120Hz, biphasic, pulse width 80 µs) has no effect on local in vivo release of dopamine, serotonin or noradrenaline during 5 hours of stimulation. This contrasts with our hypothesis but is in accordance with a recent neurochemical study that showed no alterations in tissue concentrations of dopamine and serotonin and their metabolites after stimulation of the NAc core (Sesia et al.2010).

In the patient population rapid symptomatic changes have been observed following DBS in the NAc (Denys et al.2010) and a neuroimaging study showed activation of various brain regions following acute DBS in the ventral capsule/ventral striatum in OCD patients (Rauch et al.2006). Furthermore there are several studies in rats which show rapid effects of DBS in the NAc on different behavioural paradigms (Mundt et al.2009;Sesia et al.2008;van Kuyck et al.2003;van Kuyck et al.2008). Region-specific alterations in local field potential oscillations and evoked responses in the medial PFC, lateral OFC, mediodorsal thalamus and the NAc during 90 minutes of DBS in the NAc core were reported in an electrophysiological study in rats (McCracken and Grace2009). Specifically they found an increase in beta power as well as in gamma power in the NAc. This shows that DBS in the NAc core can have rapid effects locally and in associated areas. Our study suggests the absence of a similar fast response of DBS on local monoamine release in the NAc core. Consequently, the rapid symptomatic changes in mood and anxiety appear not to result from changes in monoamine release. However, this interpretation has to be taken carefully because no tests for effects on mood or anxiety were performed in this neurochemical study. Results from in vitro studies suggested that local monoamine release could be evoked by stimulation. However these studies are performed with stimulation parameters that are not clinically relevant such as short stimulation trains of 50 pulses or less, long pulse widths of 4 ms or high intensities such as 10 mA or 20 V (Davidson and Stamford1993;Trout and Kruk1992;Kennedy et al.1992). In another electrophysiological
study a reduction in the mean firing rate of OFC neurons after 30 minutes of DBS in the NAc core in anesthetized rats was observed (McCracken and Grace 2007). This suggests that the effects of DBS may originate in other brain areas associated with the NAc core. However at this moment it is unclear whether these effects on oscillations also incorporate alterations in monoaminergic release and for that reason it would be interesting to see what the effect of DBS in the NAc core would be on the monoamine release in connected areas such as the OFC.

There are some limitations to our study. First, this study has been performed in normal healthy rats. Consequently these rats have no deviation in their monoamine levels. It is possible that the rapid effects of DBS in patients are a result of normalization of monoamine levels and are for that reason not detected in our study. Second, while this study shows that five-hour unilateral stimulation in the NAc core on one day is insufficient to elicit an effect on the local monoamine release, it may be that changes in local monoamine release are involved in the long-term effects of DBS. Therefore it is possible that repeated or longer stimulation is needed to see an effect on monoaminergic activity. Third, the effect of DBS on monoamine release may be so transient that half hour sampling time may have diluted any possible effects. However, another study which evaluated monoamine changes in the NAc core following DBS stimulation of the subthalamic nucleus had a sampling time of 20 minutes. They reported increases in release that lasted as long or longer as the duration of stimulation (Winter et al. 2008). Fourthly, a study by Meissner et al shows effects of stimulation only after administrating reuptake blockers (Meissner et al. 2003). Therefore, the absence of changes in monoamine release in this study may be explained by the failure of NAc core stimulation to counteract the effect of monoamine reuptake in the synapse. However, in a number of other studies effects of DBS on monoamine transmitter efflux were observed without uptake inhibition (Bruet et al. 2001; Hamani et al. 2010; Navailles et al. 2010). In addition, it is known that the insertion of microdialysis probes may lead to tissue damage, which has been claimed to affect local transmission and measurements of release. This may have disturbed the interaction between stimulation and local neurotransmitter release. However, the stimulation electrode was placed at a distance of 0.5 mm of the dialysis probe and previous electrophysiological studies have shown that neuronal activity and pharmacological responsiveness at that distance is not affected (van Duure et al. 2007; West et al. 2002). Moreover, microdialysis studies have been shown to reflect active neuronal release that is sensitive to local inhibition of Na-channels by tetrodotoxin (Westerink et al. 1987) or the absence of Ca-ions (Imperato and Di 1984). Lastly, there is a large variation in the stimulation parameters used in the literature. Although our parameters are at the high end of the described spectrum with 300 and 400 µA, 120Hz and a pulse width 80 µs, there was no effect of the stimulation on the behaviour of the animal or the in vivo monoamine release. However, lower amperage could possibly have a different effect than the amperages used in this study.
In conclusion, our study shows that unilateral DBS in the NAc core has no effect on in vivo monoamine release in the stimulation area. This suggests that the rapid effects of DBS in the NAc are not a result of changes in local monoamine release in the NAc core but may result from changes in other brain areas connected to the NAc. Though DBS is already widely implemented in clinical settings, the lack of knowledge of the mechanism of action of DBS prevents the full potential of its potential application. We need a better understanding of the direct and indirect neurochemical processes in the regions receiving stimulation as well as those connected and in close vicinity. Furthermore, with the emergence of DBS as an effective treatment in psychiatric patients we may eventually have to change or view on the underlying mechanism of these diseases and possibly conclude that alterations in monoamine activities are not the underlying cause of psychiatric diseases but a result of the pathology.
References


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