Understanding deep brain stimulation in obsessive compulsive disorder: A preclinical study into the mechanism of action and behaviour
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Chapter 6

Deep brain stimulation affects conditioned and unconditioned anxiety in different brain areas.

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Submitted
Abstract

Deep brain stimulation (DBS) of the Nucleus Accumbens (NAc) has proven to be an effective treatment for therapy refractory obsessive compulsive disorder. Clinical observations show that in particular anxiety symptoms decrease rapidly following DBS. Since in clinical studies different regions are targeted (nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), the internal capsule (IC)), it is of principal interest to understand which brain area is responsible for the anxiolytic effect and whether high frequency stimulation of different areas differentially affect unconditioned (innate) and conditioned (learned) anxiety.

In this study we examined the effect of high frequency stimulation in five different brain areas in rats. (NAc core and shell, BNST, IC and the ventral medial caudate nucleus (CAU)). The elevated plus maze was used to test the effect of stimulation on unconditioned anxiety, the Vogel conflict test for conditioned anxiety, and an activity test to investigate the effect of stimulation on general locomotor behaviour.

We found different anxiolytic effects of stimulation in the five target areas. Stimulation of the CAU decreased both conditioned and unconditioned anxiety, while stimulation of the IC uniquely reduced conditioned anxiety. Remarkably, neither the accumbens nor the BNST stimulation affected conditioned or unconditioned anxiety. Locomotor activity increased with NAc core stimulation but decreased with the BNST.

These findings suggest that (1) DBS may have a differential effect on unconditioned and conditioned anxiety depending on the stimulation area, and that (2) high frequency stimulation of the IC exclusively reduces conditioned anxiety. Anxiolytic effects of DBS seen in OCD patients may not be induced by stimulation of the NAc, but rather by the IC.
6.1 Introduction

Deep brain stimulation (DBS) of the nucleus accumbens (NAc), ventral striatum/ventral capsule and the anterior internal capsule has proven to be an effective treatment for therapy-refractory obsessive compulsive disorder (OCD) (Denys et al.2010; Gabriels et al.2003; Greenberg et al.2010). Clinical observations show that in particular anxiety symptoms decrease rapidly with stimulation (Denys et al.2010; Gabriels et al.2003; Greenberg et al.2010). Interestingly, reduction of anxiety in OCD patients is restricted to the symptomatic fear associated with their obsessions and is not related to unconditioned anxiety (innate fears) or general anxiety, suggesting a focused effect of DBS on conditioned anxiety (learned fear). Conditioned anxiety in animals is elicited when a certain neutral stimulus is associated with a fearful event, for example by pairing the stimulus with an electric shock while unconditioned anxiety is described as a natural behavioural reaction to a fear-evoking situation/ threat (e.g. heights or predators) at a particular moment of time (Belzung and Griebel2001; Gross and Canteras2012; Millan2003). On the basis of clinical findings, we hypothesize that stimulation of the NAc uniquely affects conditioned anxiety and not unconditioned anxiety.

This study examines the effect of stimulation in five different brain areas (NAc core and shell, the bed nucleus of the stria terminalis (BNST), the internal capsule (IC) and the ventral medial caudate nucleus (CAU)). These five brain areas are chosen on grounds of their use in human DBS studies. We used two specific behavioural paradigms for rodents to test the effect of stimulation on anxiety. First, we used the elevated plus maze (EPM) to test the effects on unconditioned anxiety. In the EPM-test a conflict is created between the natural behaviour to explore novel environments and the natural fear of heights and open spaces. Second, we used the Vogel conflict test (VCT), to test for conditioned anxiety, which is based on the conflict that water deprived rats experience when a previously acquired drinking response is ‘punished’ by a mild foot shock. In addition we used an activity metric to investigate the effect of stimulation on general locomotor behaviour in a novel environment.

6.2 Material & Methods

6.2.1 Subjects

Male Wistar rats (250-350g, Harlan, Boxmeer, the Netherlands, the NAc core and NAc shell group; Charles River, Sulzfeld, Germany, the BNST, IC and CAU group) were housed socially (2-4 animals per cage) in a temperature- and humidity controlled vivarium with a 12-h reversed light/dark cycle (lights off 7:00, on 19:00) and were provided with food and water ad libitum. Rats were handled regularly for 7 days prior to surgery by the experimenter. In total there were four consecutive experimenters, two for the NAc core and NAc shell group and two
for the BNST, IC and CAU group. The study was conducted in accordance with governmental guidelines for the care of laboratory animals and approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences.

6.2.2 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 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 as a local anaesthetic. Two bipolar electrodes (van Dijk et al.2011) consisting of two twisted platinum/ iridium wires with one pole 500 µm ventral to the other were bilaterally implanted into either the NAc core (A +1.3mm, L ±2.0mm, V 7.8mm), the NAc shell (A +1.5mm, L ±0.9mm, V 7.3), the BNST (dorsal pole medial, A -0.45mm, L ±1.5mm, V 7.2), the IC (dorsal pole anterior, A -0.8mm, L ±2.1mm, V 6.8) or the CAU (dorsal pole medial, A +1.2mm, L ±1.7mm, V 6.8). The coordinates are relative to bregma according to Paxinos and Watson (Paxinos and Watson2007). The electrodes were fixed to the skull surface with 3 stainless steel screws and dental acrylic cement. Rats were given subcutaneous finadyne (5mg/kg flunixin, Schering-Plough) post-operatively for pain management and were then housed in individual cages.

6.2.3 Experiments

After a recovery period of 7 days behavioural experiments were performed over a period of 2 weeks during the animal’s dark cycle. To find out what the effect of high frequency stimulation was on general behaviour and locomotor activity, an activity test was performed. This was followed one day later by the test for unconditioned anxiety using the EPM. In the second week the test for conditioned anxiety using the VCT paradigm was carried out (see table 1). The control groups of the NAc core, the NAc shell and the CAU group had their electrode implanted in the same target area as the stimulated group. For the VCT the control group of the NAc core and the NAc shell were combined due to technical problems surrounding the setup of the VCT. The BNST and the IC group had a joint control group in which the electrodes were implanted into the IC or the BNST. Stimulation of the rats was performed using a WPI Digital Stimulator (Model DS8000, World Precision Instruments, Sarasota, FL) and WPI Isolator (Model DLS100) connected to a 4 channel commutator (Plastics One Inc., Roanoke, VA, USA) allowing unrestricted movement of the animals in the MED skinnerbox and on the EPM. A rat was either sham stimulated, stimulated with 200µA or with 300µA (120Hz, biphasic, pulse width 80 µs).
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6.2.3.1 Activity test

A standard MED skinnerbox (30 cm in length, 22 cm in width and 50 cm in height) was equipped with a motion sensor to determine the amount of movement related activity. The recording of movement data was done by a MED interface (MED Associates, USA) connected to a personal computer running MED-PC software (MED-PC IV). After connection to the apparatus, the rat was placed in the skinnerbox for 10 minutes in which the rat was either sham stimulated, stimulated with 200µA or with 300µA (120Hz, biphasic, pulse width 80 µs) and its activity was recorded. During the experiment the levers in the MED skinnerbox were retracted and the house light was on.

6.2.3.2 Elevated plus maze

The EPM (Campden EPM 1000M) consists of two open (10 cm in width, 50 cm in length) and two enclosed arms (10cm in width, 50 cm in length, 40 cm in height) and was elevated 50 cm above the floor. The EPM was cleaned with 70% ethanol before the start of every trial. After attachment of the stimulation cable to the connector of the rat, the animal was either sham stimulated, stimulated with 200µA or with 300µA in its home cage for 10 minutes. At the end of the 10-minute period, the experimenter put the rat on the centre square of the EPM facing the same open arm for every trial. The animal was allowed to explore the EPM for five minutes while being stimulated and movement was being recorded using a video camera (Ikegami, Ikegami Electronics, Japan). After a total of 15 minutes of high frequency stimulation the rat was decoupled and transported back in its home cage. Video data was analyzed with Ethovision XT6 or XT7 (Noldus Information Technology, The Netherlands). Entry of all the three marking points (nose, center, base of tail) into an arm was scored as an event.

6.2.3.3 Vogel conflict task

In the second week of experimentation, rats were placed on water restriction, allowing one hour of drinking per day between 13.00 and 14.00. On the eighth, ninth and tenth day the animals were transported to a separate room to participate in a 15 minute experiment. These experiments were performed between 9.00 and 13.00. The animals were coupled to the stimulation cable and placed in a MED skinnerbox which was equipped with a water bottle of which the spout extended 2 cm into a drinking cavity (5 cm in width, 5 cm in height and

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3 cm in depth). The number of licks was registered by a lick-o-meter (MED ENV 250) which detects a short circuit when animals touch the tip of the spout. The floor consisted of a grid, which was connected to a MED interface including a micro controlled shock source (MED ENV 413). A transparent Plexiglas screen was used to separate the part of the skinnerbox containing the water bottle from the rest of the box. On the eighth day the rats were allowed to explore the skinnerbox and find the spout. When the first licks were registered, the rats were taken out of the skinnerbox and transported to their home cage. On the ninth day they underwent the adaptation test to establish a baseline drinking level over a period of 5 minutes. Rats were again connected to the stimulation cable and put in the skinnerbox. They were not able to approach the spout until the 10 minutes of high frequency stimulation had passed. Subsequently, the Plexiglas screen was lifted for five minutes in which they could drink while receiving high frequency stimulation. After five minutes had passed, the rats were disconnected and returned to their home cage having received a total of 15 minutes of high frequency stimulation. On the tenth day the VCT was performed. The procedure was the same as during the adaptation test except a single shock of 0.35 mA was delivered to the rat after every 20th lick.

6.2.4 Histological examination

After completion of the experiment, all rats were given an electrolytic lesion by passing 100 µA of direct current for 20 seconds to allow subsequent localization of the electrode tip. One day after lesioning, rats were euthanized with carbon dioxide in a pre-filled chamber with a CO₂/O₂. The brains were then removed and frozen. Coronal sections of 30 µm were cut on a cryostat and stained with Thionin Blue or Cresyl violet for determination of the exact location of the two electrodes.

6.2.5 Data analysis

Data is reported as mean ±SEM. The data of the activity test, EPM and VCT was analyzed for high frequency stimulation (sham, 200 and 300 µA) effects using one-way Analysis of Variance (ANOVA). P values below 0.05 (significant) or between 0.05 and 0.10 (a trend) were followed up with a Dunnett post hoc test. Behavioural measurements of animals on the EPM were scored by dividing the amount of open arm entries by the amount of closed arm entries to acquire the ratio of open arm entries (RO). The time spent on the open arms was divided by the time on the closed arms to acquire the percentage of time spent on the open arms (%TO). The centre square was not used for the analyses. Animals were considered to be in an arm when an area from the tip of the head to the base of the tail passed into an arm. The VCT data was analysed for the amount of licks during the Adaptation session and the VCT.
6.3. Results

6.3.1 Histology

Animals were included in the data analysis when histological examination showed correct bilateral placement of the electrodes in the NAc core, the NAc shell, the BNST, the IC and the CAU (fig. 1). Two animals were excluded from data analysis for the activity test due to detachment of the cable during stimulation. Eight animals were excluded from the EPM test due to practical problems with either the video recording, the stimulation wire, or when rats fell off the EPM during the test session. Due to technical problems with the VCT the animals used for data analysis in the VCT in the NAc core and some in the NAc shell group are different than the animals used in the activity test and EPM test. However all animals underwent all three behavioural tests. Twenty one animals were excluded from the data analysis of the VCT in the BNST/IC and CAU group. This was caused by rats either not approaching the water bottle during the adaptation or the VCT, losing their head caps or incorrect stimulation settings.

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Fig 1: Histology of the included animals. Localization of the electrodes in (A) the NAc core, NAc shell, (B) CAU and (C) BNST, IC.

6.3.2 NAc core

For the activity test one-way ANOVAs showed a significant effect of high frequency stimulation on mean activity in the NAc core group (F(2, 20) = 3.78, p = 0.041). A Dunnett post hoc analyses showed a significantly higher activity for the 300 µA group (7 rats, p = 0.031) compared to control (9 rats). However, the 200 µA group (7 rats) did not significantly differ from the control group. Second, there was no significant effect of high frequency stimulation with 200 µA (7 rats) or 300 µA group (8 rats) on the RO, %TO and distance moved on EPM compared to
control (8 rats). Lastly, there was no significant effect for the NAc core 200 µA group (7 rats) or
the NAc core 300 µA group (8 rats) on the total number of licks during the adaptation test and
the total number of licks during the VCT compared to control (11 rats).

6.3.3 NAc shell
Firstly, one-way ANOVAs did not reveal a significant effect for either the 200 µA (7 rats) or the
300 µA (9 rats) high frequency stimulation of the NAc shell on activity compared to control
(9 rats). Secondly, there was no significant difference for the RO, %TO and distance moved on
EPM between the control group (9 rats) and the 200 µA (7 rats) and 300 µA (8 rats) group.
Last, high frequency stimulation of 200 µA (6 rats) or 300 µA (10 rats) had no significant effect
on the total number of licks during the adaptation test or the total number of licks during the
VCT compared to control (11 rats).

6.3.4 BNST/ IC
One-way ANOVA showed a significant effect of high frequency stimulation on mean activity in
the BNST/ IC group (F(4, 48)= 7.290, p < 0.001). Activity significantly decreased in the BNST 200
µA group (9 rats, p = 0.001), the BNST 300 µA group (9 rats, p < 0.001) and a trend for the IC
300 µA group (10 rats, p = 0.053) but not in the IC 200 µA group (9 rats) compared to control
(16 rats). There were no significant differences for the RO, %TO and distance moved on EPM
between the control group (15 rats), the BNST 200 µA group (8 rats), the BNST 300 µA group
(8 rats), the IC 200 µA group (9 rats) or the IC 300 µA group (10 rats). Lastly, one-way ANOVAs
revealed a significant effect of high frequency stimulation on the total number of licks during
the VCT (F(4, 37)= 10.350, p < 0.001) but not for the adaptation test. The total amount of licks
during the VCT significantly increased in the IC 200 µA group (7 rats, p = 0.001) and the IC 300
µA group (7 rats, p = 0.001) but not in the BNST 200 (7 rats) or the BNST 300 µA group (6 rats)
compared to control (15 rats).

6.3.5 CAU
There were no significant effects of 200 µA (10 rats) or 300 µA (10 rats) high frequency
stimulation of the CAU on activity compared to control (12 rats). When comparing the EPM
results for the 200 µA group (10 rats) and 300 µA group (10 rats) to control (10 rats), one-way
ANOVAs showed a trend for %TO (F(2, 27)= 3.327, p = 0.051) but not for the RO or distance
moved. A Dunnett post hoc analyses showed a significant effect for %TO for the 300 µA group
(p = 0.039) compared to control. A significant effect of high frequency stimulation was found on
the total number of licks during the VCT (F(2, 21)= 5.833, p = 0.010) but not for the adaptation
test. There was an anxiolytic effect for the 300 µA group (6 rats, p = 0.043) on the total amount
of licks during the VCT compared to control (8 rats) but not for the 200 µA group (10 rats).
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Fig 2: Effect of high frequency stimulation in the NAc core, NAc shell, CAU, BNST and IC on (A) the mean activity as measured by a motion sensor in a MED skinner box, (B) on time spent in the open arm versus the closed arm in the EPM test and (C) on the number of licks during the VCT. * is p < 0.05 compared to control
6.4 Discussion

We investigated the effect of stimulation in two anxiety, and one activity model in five different brain regions of the rat. Stimulation of the CAU decreased both conditioned and unconditioned anxiety, and stimulation of the IC uniquely conditioned anxiety. Neither NAc nor BNST stimulation affected conditioned or unconditioned anxiety. Locomotor activity increased with NAc core stimulation but decreased with stimulation of the BNST. These findings show that (1) stimulation has a differential effect on unconditioned and conditioned anxiety depending on the stimulation area, and that (2) stimulation of the IC exclusively reduced conditioned anxiety. These findings, when translated to humans, suggest that the anxiolytic effects of DBS seen in OCD patients may not be induced by stimulation of the NAc, but rather by the IC.

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Table 1: Summary of the behavioural results

Stimulation of the CAU decreases unconditioned anxiety as measured in the EPM test and conditioned anxiety as measured in the VCT. There is currently limited preclinical research available of the role of the CAU in anxiety. Injections of somatostatin, a growth hormone-inhibiting hormone, in the CAU had no anxiolytic effect in an unconditioned anxiety paradigm (Tashev et al.2001). Rodriguez-Romaguera et al demonstrated that high frequency stimulation at the border of the NAc and the CAU enhanced the extinction of conditioned fear using an auditory fear conditioning paradigm (Rodriguez-Romaguera et al.2012). We found an anxiolytic effect of CAU stimulation in the VCT, which is comparable with the conditioning phase of the auditory fear conditioning paradigm. Both these findings suggest that the CAU may play a role in conditioned anxiety in rodents. Though clinical evidence for a direct link between the CAU and anxiety is currently lacking, imaging studies consistently show abnormalities of the CAU in OCD (Adams et al.2005;Breiter et al.1996;Lucey et al.1997;Rauch et al.1994;Whiteside et al.2006). Moreover, Aouizerate et al showed that DBS of the CAU markedly improved anxiety symptoms in one OCD patient (Aouizerate et al.2004). To conclude, our results suggest a role for the CAU as stimulation target for decreasing unconditioned as well as conditioned anxiety.
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Stimulation in the anterior part of the IC had an anxiolytic effect on conditioned but not on the unconditioned anxiety. To our knowledge, this is the first preclinical study that investigates the impact of stimulation on anxiety in the IC. In clinical practice, electrodes targeted at the NAc and VC/VS region have been shown to have the most optimal responder rate when the contact points of the electrode in the IC are activated (de Koning et al. 2011). Moreover, clinical experience clearly shows that the anxiolytic effect of DBS in OCD patients solely affects the OCD related conditioned anxiety that is the subject of obsessions and compulsion and not the general, unconditioned anxiety. This suggests that stimulation of the IC has a unique anxiolytic effect on conditioned anxiety, and corroborates the hypothesis that the effect of DBS is due to activation of axonal fibers running through the ventral part of the IC (Lehman et al. 2011). In rodents the IC is located at a more posterior position than in humans, separating the BNST and the Globus Pallidus. Stimulation of cortico-fugal tracts running along the IC may affect the PFC. The PFC is known to be involved in anxiety (Duncan et al. 1996) and in controlling anxiety and conditioned fear (de Visser et al. 2011; Graham and Milad 2011; Kim et al. 2011). Though indirect evidence, Rodriguez-Romaguera et al showed in rats that high frequency stimulation of the CAU increased cell plasticity in the mPFC as well as the OFC (Rodriguez-Romaguera et al. 2012). This may be due to activation of white matter fascicules that are running through the CAU and are part of the cortico-fugal fiber bundles. To summarize, our findings show a clear link between stimulation of the IC and a decrease of conditioned anxiety, which may explain the anxiolytic effects observed in OCD patients with activation of electrodes in the IC targeted at the NAc.

The absence of anxiolytic effects of BNST and NAc stimulation in the EPM test as well as the VCT is surprising. The lack of effect in the BNST, which anatomically is located near to the IC demonstrates that the effect of stimulation is highly localized. Though inconsistent, some studies using c-fos expression, local pharmacological injections or lesions show involvement of the NAc shell and the BNST in the EPM test (da Cunha et al. 2008; Duncan et al. 1996; Gomes et al. 2011; Horsley et al. 2007; Muigg et al. 2009; Sahuque et al. 2006; Silveira et al. 1993; Treit et al. 1998; Waddell et al. 2006). Although these studies suggest that manipulation of the NAc and the BNST may have an anxiolytic effect, our study clearly shows that electrical stimulation of these two areas does not. The lack of effect in our study may be due to the type of electrode used, the stimulation parameters, the length of stimulation or the anxiety paradigms. The BNST for example is more associated with chronic, diffuse anxiety (Davis et al. 2010; Walker et al. 2003) while the EPM and VCT both measure more acute forms of anxiety. It would be of interest to assess the effects of stimulation targeted at the BNST in paradigms that produce more long-term anxiety, such as contextual conditioning.
We investigated the effect of stimulation on general behaviour/locomotor activity in a novel environment. Stimulation of the NAc core resulted in higher activity while stimulation of the BNST reduced activity. It is known that the NAc is involved in spontaneous and drug-induced psychomotor activation (Donzanti and Uretsky 1983; Hamilton et al. 1986; Kelley et al. 1989; Pijnenburg et al. 1975) whereas the role of the BNST in locomotor activity has been less thoroughly investigated. Two studies showed that electrical lesions or pharmacological inactivation of the BNST did not result in a change in activity in a novel environment (Pezuk et al. 2008; Wenzel et al. 2011). The fact that we see an effect on locomotor activity and not on anxiety while stimulating the NAc and the BNST suggest that there is no link between the two conditions. This implies a high degree of specificity on the observed rodent behaviours influenced by stimulation.

There are some limitations and possible confounding factors in the EPM test as well as in the VCT. Firstly, we observed that high frequency stimulation may affect locomotor activity which can have an impact on the outcome measurements in the EPM. However, measurements of total distance moved during the EPM test (reflecting locomotor activity) were not influenced by high frequency stimulation in any of the five target areas. Therefore, the effect of high frequency stimulation in the CAU in the EPM test cannot be explained by effects on locomotor activity and reflects a decrease in unconditioned anxiety. Secondly, it could be suggested that the effect measured in the VCT is not an anxiolytic effect but is due to a change in pain sensitivity or an increased motivation to drink. However, rats treated with morphine, in a dose that significantly increased the pain threshold, did not show an increase in licks in the VCT which contradicts that an increase in punished licks is due to an analgesic effect (Agmo et al. 1991; Basso et al. 2011). Several studies reported that there is no effect on the number of licks when the rats have an extended water deprivation and are thus more motivated to drink (Agmo et al. 1991; Tonetto et al. 2009). Our results show no effect of high frequency stimulation on the number of licks during the adaptation phase which corroborates these findings. Hence the effect of high frequency stimulation cannot be explained by variations in nociceptive threshold or motivation to drink but can be ascribed to an anxiolytic effect of high frequency stimulation in the IC and the CAU on conditioned anxiety. Thirdly, a common limitation of investigating the effect of stimulation in rodents is the anatomical differences with humans. For example, in humans, the IC separates the CAU and the putamen through a broad band of white matter, while in rodents this band is absent. The IC in rodents is located at a more posterior position separating the BNST and the Globus Pallidus which hinders the extrapolation of results obtained in rodents to humans. In addition, a different nomenclature is used to describe the various clinical DBS targets -NAc, ventral striatum/ventral capsule and the anterior IC-, while it is possible that stimulation of the same anatomical area underlies
their efficacy. Fourthly, we report effects in rats that were stimulated ten minutes before and during the behavioural tests. It could be suggested that chronic stimulation might have resulted in a different effect. However, the effect of DBS on anxiety in patients is acute and can be seen within minutes after the onset of stimulation and is maintained as long as the stimulation continues. Lastly, a notable difference is the variation between the control groups during the VCT (control group CAU compared to control group NAc). The animals used for the NAc core and NAc shell group came from a different supplier than the CAU group. The variations between the control groups can be caused by difference in experimenters but are more likely to be due to the different origin of the animals.

In conclusion, DBS in OCD patients has shown to have very rapid effects on anxiety. However, neither the type of anxiety nor the precise target has been elucidated in the clinical trials. Though the present studies were carried out in rats, the results indicate that depending on the precise target, different types of anxieties may be attenuated. Our findings, extrapolated to humans, suggest that the anxiolytic effect of DBS seen in OCD patients is due to stimulation of the IC and/or the CAU and not the target area the NAc. These results may help to identify the best target for DBS effects on fear and anxiety.
Chapter 6

References


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