Understanding deep brain stimulation in obsessive compulsive disorder: A preclinical study into the mechanism of action and behaviour
van Dijk, Addy

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)
Chapter 7

The impact of deep brain stimulation on compulsive grooming in sapap3 mutant mice

Addy van Dijk, Matthijs G.P. Feenstra, Gouping Feng, Damiaan Denys

In preparation
Chapter 7

Abstract

Obsessive compulsive disorder (OCD) is a psychiatric disorder affecting 2% of the population. Deep Brain Stimulation (DBS) of the nucleus accumbens, ventral striatum/ventral capsule and the anterior internal capsule has proven to be an effective treatment for these patients. Yet, there remain many questions regarding the optimal target for DBS, the mechanism of action and the specific impact of DBS on the different symptoms of OCD.

In this study, we examined the effects of DBS on compulsive grooming behaviour of the sapap3 mutant mouse, an animal model for OCD. Sapap3 mutant mice were bilaterally stimulated at 3 brain areas - the nucleus accumbens (NAc), the internal capsule (IC) and the bed nucleus stria terminalis (BNST) for 2 hours. Stimulation of the BNST had no effect on compulsive grooming behaviour or locomotor activity. Stimulation of the NAc and the IC did not alter the percentage of time spent grooming but did increase the number of times grooming is initiated (i.e. number of bouts). This resulted specifically in the IC in a significant decrease of the duration of bouts, implying that IC stimulated sapap3 mutant mice initiated the grooming behaviour more often but that the grooming bout was aborted quicker. Stimulation of the NAc but not the IC increased the distance moved and the movement velocity of the sapap3 mutant mice.

These results in combination with earlier findings of an anxiolytic effect of IC stimulation on conditioned anxiety suggest an important role of the IC in the working mechanisms of DBS
7.1 Introduction

Obsessive compulsive disorder (OCD) is a psychiatric disorder affecting 2% of the population. OCD is characterised by obsessive thoughts, resulting in anxiety and by compulsive actions reducing anxiety. About 10% of OCD patients remain therapy-resistant (Denys 2006). Deep Brain Stimulation (DBS) of the nucleus accumbens, ventral striatum/ventral capsule and the anterior internal capsule has proven to be an effective treatment for these patients (Denys et al. 2010; Gabriels et al. 2003; Greenberg et al. 2010). Yet, there remain many questions regarding the optimal target for DBS, the mechanism of action and the specific impact of DBS on the different symptoms of OCD.

In this study, we examined the effects of DBS on compulsive grooming behaviour of the sapap3 mutant mouse, an animal model for OCD (Welch et al. 2007). The absence of sapap3, a postsynaptic scaffolding protein that is highly expressed at excitatory striatal synapses, results in compulsive grooming, increased anxiety and impaired corticostriatal transmission, a neurobiological hallmark of OCD (Welch et al. 2007). We hypothesized that DBS is capable of altering compulsive grooming of sapap3 mutant mice.

Therefore, sapap3 mutant mice were bilaterally stimulated at 3 brain areas - the nucleus accumbens (NAc), the internal capsule (IC) and the bed nucleus stria terminalis (BNST) for 2 hours after which we analysed the percentage of time the mice spent on grooming, the number of times grooming is initiated (i.e. number of bouts), the bout duration and the locomotor activity of the mice.

7.2 Materials & Methods

7.2.1 Subjects

7.2.1.1 NAc group

The experiments of the NAc group were performed at the Department of Neurobiology, Duke University Medical Center, Durham, USA. Sapap3 mutant mice (Welch et al. 2007) were housed socially 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. Genotypes were determined by PCR of mouse tail DNA, using primer F1 (ATTGGTAGGCAATACCAACAGG) and R1 (GCAAAGGCTCTTCATATTGTTGG) for the wild-type allele (147 base pairs), and F1 and R2 (CTTTGTGGTTCTAAAGTACTGTGG; in neo cassette) for the mutant allele (222 base pairs). All experiments were performed during night time (dark) and food and water were available ad libitum. All animal procedures were done according to protocols approved by the Institutional Animal Care and Use Committee of Duke University.
Chapter 7

7.2.1.2 BNST and IC group
The experiments of the BNST and IC group were performed at the Netherlands Institute for Neuroscience, Amsterdam, the Netherlands. Sapap3 mutant mice (Welch et al. 2007) were housed socially 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. Genotyping was performed by real-time PCR assays using the same primers as the NAc group. All experiments were performed during day time (light) and food and water were available ad libitum. The study was conducted in accordance with governmental guidelines for care of laboratory animals and approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, the Netherlands.

7.2.2 Phenotyping
The sapap3 mutant mice used for the BNST and IC group were videotaped for 2 hours (12:00-14:00) during the light period. They were analyzed for grooming behaviour by a trained observer with the use of Ethovision XT6 and 7 (Noldus Information Technology, The Netherlands). We looked at the percentage of time the mice spent grooming and the number of times the mice started the grooming behaviour (i.e. the number of bouts). Mice that groomed at least 12% of the time were included into the DBS experiments.

7.2.3 Surgery
On the day of the surgery the mice were anesthetized with isoflurane in O2:air (1:1). Pre-operatively Metacam (1 mg/kg Meloxicam, Boehringer Ingelheim) was given subcutaneous for pain management. The animals were then placed in a Kopf stereotactic apparatus (Kopf Instruments, Tujunga, CA) and kept at a constant temperature of 37°C using a rectal thermometer connected to a heating pad. A small incision was made to expose the skull after which Xylocaine (Lidocaine 10% spray, AstraZeneca BV) was used for local anaesthesia. Two bipolar electrodes were bilaterally implanted into the NAc (A+1.5mm, L±0.9mm, V4.8mm), the BNST (10° in the coronal plane, A-0.25mm, L±1.44mm, V4.3) or the IC (A-0.4, L±1.25mm, V4.2). The coordinates are relative to bregma according to Paxinos and Watson, 2007 (Paxinos and Watson 2007). The bipolar electrode consisted of two twisted 60 µm platinum/iridium wires with one pole 500 µm ventral to the other for the NAc group and the two poles next to each other for the BNST and IC group. The electrodes were fixed to the skull surface with dental acrylic cement (Flowline, Heraeus Kulzer; Optibond FL, Kerr). Mice were housed in individual cages after surgery.

7.2.4 Experiments
After a recovery period of 7 days, behavioural experiments were performed. Mice underwent 3 experimental days with at least one resting day in between. On the experimental days, the
mice were attached to the DBS cable in their homecage through a commutator allowing free unrestricted movement of the animals. After two hours the stimulation was turned on by using a WPI Digital Stimulator (Model DS8000, World Precision Instruments, Sarasota, FL) and a WPI Isolator (Model DLS100). A mouse was either, in random order over the three experimental days, sham stimulated, stimulated with 200 or 300µA (120Hz, biphasic, pulse width 80 µs) for 2 hours and videotaped to allow behavioural analysis. The two hours of stimulation were analyzed for grooming behaviour by a trained observer who was blind to the stimulation condition and the locomotor activity of the mouse was analyzed with the use of Ethovision XT6 and 7 (Noldus Information Technology, The Netherlands). Locomotor activity was determined as distance moved and movement velocity while grooming behaviour was analyzed for the percentage of time the mice spent grooming and the number of times grooming was initiated (i.e. number of bouts). The bout duration was calculated by dividing the total amount of time in seconds spent grooming divided by the total number of bouts. The NAc group was observed from 01:00 till 03:00 in the dark period while the BNST and IC group were observed in the light period from 12:00 till 14:00.

7.2.5 Control experiment

An additional experiment was performed to control for natural variations in the grooming behaviour of the sapap3 mutant mice. The mice of the NAc group underwent three control experimental days instead of one with at least one resting day in between. On these three days the mice were attached to the DBS cable in their homecage without being stimulated (sham stimulation). The control group was observed from 01:00 till 03:00 in the dark period for grooming behaviour by a trained observer.

7.2.6 Histological examination

After completion of the experiment, all mice were given an electrolytic lesion of 200 µA direct current for 20 seconds to allow subsequent localization of the electrode tip. One day after lesioning, mice were euthanized with carbon dioxide in a chamber pre-filled with a CO₂/O₂. The brains were then removed and frozen. Coronal sections of 30 μm were cut on a cryostat and stained with Cresyl violet for determination of the exact location of the two electrodes.

7.2.7 Data analysis

Data is reported as mean ± SEM. Statistical analyses of the percentage of time spent grooming, the amount of bouts, the bout duration, distance moved and velocity were performed with a Repeated Measures Analysis of Variance (RM-ANOVA), with stimulation parameters as ‘within subjects’ factor. If indicated by the Mauchly’s test of sphericity, the number of degrees of freedom were adjusted by a Greenhouse-Geisser correction. If appropriate this was followed by a test of a simple within-subjects contrasts analysis in which the 200 and 300 µA stimulation were compared to sham stimulation.
Chapter 7

7.3 Results

7.3.1 Inclusion

Animals were included in data analysis when the histological examination showed correct bilateral placement of the electrodes in the NAc, the BNST and the IC (fig. 1). The NAc group consisted of 9 sapap3 mutant mice, the IC of 8 sapap3 mutant mice and the BNST of 4 sapap3 mutant mice. The BNST group was not explored further after the first 4 animals due to the absence of an effect of DBS.

Only mice with a presurgery grooming percentage of approximately 12% or higher were used for the DBS experiment. However, the percentage of time and the number of bouts were limited due to attachment of the mice to the DBS cable. This explains the difference between the stated inclusion criteria of 12% and the reported percentages between 6 and 11% for the DBS experiments.

7.3.2 NAc group

We found no significant difference between stimulation and sham stimulation for the percentage of time spent grooming and the bout duration. However stimulation of the NAc had a significant effect on the total amount of bouts ($F = 4.945, p = 0.021$). Test for within-subjects contrasts showed that the 300 µA stimulation resulted in 130.3 bouts, which is a significant increase in bouts compared to sham stimulation ($79.4, F = 9.469, p = 0.015$). Furthermore stimulation of the NAc caused an increase in distance moved (200 µA: $F = 14.934, p = 0.029$; 300 µA: $F = 5.913, p = 0.059$) and velocity (200 µA: $F = 17.809, p = 0.008$; 300 µA: $F = 7.706, p = 0.039$) compared to control.

Fig 1: Histology of the included animals. Localization of the electrodes in (A) the NAc, (B) BNST and IC.
7.3.3 BNST group
Statistical analysis showed no effect of stimulation in the BNST on activity or time spent grooming, total amount of bouts or bout duration during 2 hours of stimulation with either 200 or 300 µA.

7.3.4 IC group
There was no significant effect of stimulation in the IC on the percentage of time spent grooming. However IC stimulation had a significant effect on the total amount of bouts (F = 4.600, p = 0.029) and showed a trend for the bout duration (F = 2.790, p = 0.096). The 300 µA stimulation increased the total amount of bouts from 38.8 (sham stimulation) to 64.8 (F = 11.720, p = 0.011) and decreased the duration of bouts from 13.7 s to 7.1 s (F = 8.101, p = 0.025). Distance moved or velocity were not altered by IC stimulation.

7.3.5 Control experiment
There was no significant difference between the percentage of time spent grooming, the total amount of bouts or bout duration during the three control days.

Fig 2: Effect of high frequency stimulation in the NAc, BNST and The IC on (A) the percentage of time spent grooming, (B) the duration of one bout, (C) the total number of grooming bouts and (D) the velocity. * is p < 0.05 compared to control.
7.4. Discussion

We investigated the effect of DBS on compulsive grooming in three brain areas – the NAc, the IC and the BNST- in the sapap3 mutant mice. Stimulation of the BNST had no effect on compulsive grooming behaviour or locomotor activity. Stimulation of the NAc and the IC did not alter the percentage of time spent grooming but did increase the number of times grooming is initiated (i.e. number of bouts). This resulted specifically in the IC in a significant decrease of the duration of bouts, implying that IC stimulated sapap3 mutant mice initiated the grooming behaviour more often but that the grooming bout was aborted quicker. Stimulation of the NAc but not the IC increased the distance moved and the movement velocity of the sapap3 mutant mice.

The absence of a stimulation induced effect on the percentage of time spent grooming is in contrast with three previously published studies that have reported changes of compulsive behaviour following NAc and/ or BNST stimulation. The first study showed that low frequency stimulation of the NAc increased compulsive behaviour in a T-maze following administration of 8-OH-DPAT in rats (van Kuyck et al.2003). A second paper of the same group demonstrated that high frequency stimulation in the NAc or the BNST reduced compulsive drinking in the schedule induced polydipsia model (van Kuyck et al.2008). Lastly, high frequency stimulation in the NAc core and shell reduced quinpirole-induced compulsive checking in rats (Mundt et al.2009). These studies clearly demonstrate that high frequency stimulation of the NAc and the BNST, independent of the paradigm, may reduce compulsive behaviour. The contrast between our results and these studies may be explained by different reasons. The mode of compulsivity in the animals, induced either by pharmacological injections or by food restriction, is only present during the limited time of exposure of the chemical or behavioural inductor and differs clearly from a genetic animal model in which the compulsive grooming is constantly present. In addition, different types of compulsion are being explored - checking, drinking and grooming-. Possibly the discrepancy between the results reported here and the three studies mentioned above is due to these differences in the paradigms used. Finally, there is a large variation in the electrodes and stimulation parameters used in the literature. For example, our parameters are at the high end of the described spectrum with 300 µA. Lower amperage could possibly have a different effect than the amperages used in this study.

Another parameter that may be used to describe the compulsive behaviour of these mice is the number of grooming bouts in addition to the percentage of time spent grooming (Welch et al.2007). In this study, we examined also a third parameter of compulsivity called the duration of bouts. In humans, decrease of compulsivity may be observed as the reduction of the total time of the compulsive behaviour, in the number of times that a person engages in compulsive action, and the duration of each compulsive action. The latter illustrates that
patients are capable to inhibit faster each separate compulsive action. Interestingly, we observed a significant impact of NAc and IC stimulation on these last two parameters. NAc and IC stimulated mice initiated the grooming behaviour more often (i.e. increased total number of bouts). This only led to a significant decrease in duration of bouts in the IC stimulated group. Thus, grooming was more fragmented during IC stimulation: it was more rapidly aborted, but also more often initiated. A possible explanation for this finding could be that IC stimulation increases the capability to inhibit a separate compulsive action faster. There is one other study that investigated bout duration in compulsive behaviour (Hoffman and Rueda Morales2012). In this study, D₁ and D₂ dopamine receptor antagonists (Raclopride and SCH23390) decreased bout duration without altering individual bout components or the total number of bouts in compulsive nest building of pregnant rabbits (Hoffman and Rueda Morales2012). The authors hypothesized that dopamine alters the motivation of the rabbit to maintain the compulsive nest building for an extended period of time. It is known that dopamine is related to processes that underlie the motivation to execute certain behaviours (Wise2004). At this moment, possible effects of IC stimulation on dopamine release are unknown. To conclude, stimulation of the NAc and the IC alters the grooming behaviour in the sapap3 mutant mice which only in the IC stimulated group led to a reduction in the duration of a grooming bout. Possibly, stimulation of the IC alters dopamine transmission, which might decrease the motivation to maintain the compulsive grooming during an individual grooming bout.

Our observation that stimulation in different brain areas affects compulsive grooming behaviour could be of clinical significance. In clinical practice we observe that with DBS anxiety in OCD patients rapidly decreases followed by changes in compulsive behaviour within weeks to months (Denys et al.2010). In a previous study we found an anxiolytic effect of DBS in the IC on conditioned but not on the unconditioned anxiety. However neither the NAc nor the BNST stimulation had an anxiolytic effect (van Dijk et al, 2012 in preparation). In the present study we describe a decrease in duration of bouts after IC but not NAc stimulation. In humans, electrodes implanted in the NAc and VC/VS region have been shown to have a higher responder rate if the contact points of the electrode nearer toward the IC are activated (de Koning et al.2011). The fact that IC stimulation uniquely affects conditioned anxiety and shortens the grooming bout combined with clinical data suggests that stimulation of the IC might be the underlying cause of the efficacy of DBS.

There are some limitations to our study. First, The NAc group was stimulated during the dark period while the IC and BNST group were stimulated during the light period. This could possibly have had an influence on the grooming behaviour. However stimulation in the NAc and the IC had the same effect on the total number of bouts while the groups were not stimulated in the same dark/ light period. Second, when we compared the grooming behaviour between
sham stimulated animals (attached to the DBS cable) and freely moving operated animals, we observed that the percentage of time and the number of bouts were reduced (17.80 to 9.18 %; 133 to 45). This is likely due to attachment of the mice to the cable and shows that being tethered influences the grooming behaviour. Despite this influence we find a significant difference between the stimulated and sham stimulated animals in grooming behaviour. Third, in this study the observer only determined whether the mouse was actively grooming or not (start-stop scoring). This allowed us to analyze the percentage of time the mice spent grooming and the number of times grooming was initiated (i.e. number of bouts). However, grooming behaviour is more complex. It occurs in a particular stereotyped order of four phases (Berridge et al.2005). Possibly by simplifying the scoring of the grooming behaviour in this study, effects of DBS on the different grooming phases could have been overlooked. Fourth, a limitation of investigating the effect of stimulation in rodents is the anatomical difference 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. Last, we report effects of 2 hour stimulation in the sapap3 mutant mice. In OCD patients, compulsions take weeks to months to improve after the onset of stimulation. This might suggest that chronic stimulation is needed to see an effect of stimulation on the percentage of time spent grooming in these mice. Future studies, using stimulation periods of at least a couple of days should provide a better comparison with the effects of clinical chronic stimulation.

In conclusion, high frequency stimulation in the IC, but not in the NAc or the BNST, reduces the duration of compulsive grooming bouts in the sapap3 mutant mice. These results in combination with earlier findings of an anxiolytic effect of IC stimulation on conditioned anxiety suggest an important role of the IC in the working mechanisms of DBS. DBS has proven to be an effective treatment for OCD patients. However, the precise anatomical target that underlies its efficacy has not been elucidated in the clinical trials. These results may help to identify the best target for DBS in OCD patients.
References


