Understanding deep brain stimulation in obsessive compulsive disorder: A preclinical study into the mechanism of action and behaviour
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Chapter 8

Summary and general discussion
8.1 Summary and General discussion

DBS of the nucleus accumbens and ventral striatum/ventral capsule has proven to be an effective treatment for patients with OCD (Denys et al. 2010; Gabriels et al. 2003; Greenberg et al. 2010). DBS has a rapid effect on anxiety and mood followed by changes in compulsive behaviour within weeks to months (Denys et al. 2010). Yet, there remain many questions regarding the optimal target for DBS, the neurobiological mechanisms underlying the rapid effects of DBS and the specific impact of DBS on the different symptoms of OCD. In this thesis, we report a series of experiments in rodents addressing these questions. The aim of the thesis was two-fold: (1) to examine the mechanism of action of DBS, i.e. the neurobiological underpinnings such as changes in monoamine levels and neurogenesis, and (2) to examine the differential effect of DBS on anxiety and compulsivity, both core symptoms of OCD.

8.2 Summary of main findings

The mechanism of action of DBS

There is compelling evidence that the serotonergic system plays a major role in the treatment of OCD (chapter 2). Pharmacotherapeutic studies have shown that SSRIs are more effective in achieving clinical response compared to placebo, and that atypical antipsychotics, which have a high affinity for 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors, may augment the effect of SSRIs. However, these findings do not necessarily reflect the existence of a neurobiological abnormality in the central serotonergic system in OCD. At this moment, direct evidence for an abnormality in the serotonergic system in the pathophysiology of OCD is still scarce. Hence, it is open to discussion whether the serotonergic system plays a primary role in the pathophysiology of OCD or is secondarily involved due to the efficacy of SSRIs. SSRIs may modulate serotonin via an intact serotonergic system to compensate for the underlying pathogenesis that is related to another neurotransmitter system. Several reviews have summarized the growing evidence that dopamine might be involved in OCD as well (Denys et al. 2004c; Koo et al. 2010). For example, atypical antipsychotics with potent 5-HT\textsubscript{2A}, 5-HT\textsubscript{2C} and D\textsubscript{2} antagonistic properties have antiobsessional properties as additional therapy to SSRIs. Denys et al. found that the combination of quetiapine and fluvoxamine may cause a synergistic dopamine increase in the prefrontal cortex and thalamus (Denys et al. 2004a). Moreover, SPECT and PET studies in OCD patients hint to decreased D\textsubscript{2} receptor binding in the ventral striatum (Denys et al. 2004b), dopamine transporter binding abnormalities in the basal ganglia (Kim et al. 2007; van der Wee et al. 2004) and indicate that successful SSRI treatment is associated with normalization of the dopamine function (Kim et al. 2007; Pogarell et al. 2005). Therefore, future research should focus much more on the interaction of the serotonergic system with other neurochemical systems, such as dopamine in relation to OCD.
In **chapter 3 and 4** we report the effect of DBS in the NAc on monoamine levels locally and in the prefrontal cortex. Using freely moving animals we found that DBS of the NAc core has no effect on local in vivo release of dopamine, serotonin or noradrenaline. However, we found 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 following onset of stimulation in the NAc core. Our study suggests that activation of monoamine release in the prefrontal areas rather than local effects in the NAc core are involved in the early behavioural effects of DBS in the NAc.

In **chapter 5** we found no effect of DBS of the NAc on neurogenesis in the dentate gyrus (DG) during stimulation or 5 days following stimulation. Our results contrast with other studies, showing profound effects of high frequency stimulation on neurogenesis in the DG after stimulation in the anterior nucleus of the thalamus, subthalamic nucleus and the entorhinal cortex (Encinas et al.2011;Khaindrava et al.2011;Stone et al.2011;Toda et al.2008). The lack of effect could be due to an absence of projections of the NAc to the DG or to acute nature of the stimulation. Our preliminary results suggest that hippocampal neurogenesis is not involved in the therapeutic effects of DBS in the NAc.

**The effect of DBS on anxiety and compulsivity**

In **chapter 6** we explored the effect of DBS in five different brain areas (NAc core and shell, BNST, IC and CAU) on *unconditioned* and *conditioned* anxiety. 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 NAc nor BNST stimulation affected conditioned or unconditioned anxiety. 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 in OCD patients may not be induced by stimulation of the NAc, but rather by the IC.

The effect of DBS on compulsive grooming of the sapap3 mutant mouse, an animal model for OCD, was examined in **chapter 7**. Sapap3 mutant mice were bilaterally stimulated at 3 brain areas, the NAc, IC and 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 in the IC specifically, 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.
8.3 General discussion

The mechanism of action of DBS

To our knowledge we are the first who have investigated the effect of DBS of the NAc core on in vivo monoamine release. Stimulation of the NAc core had no effect locally on serotonin, dopamine or noradrenaline levels. Although some in vitro studies suggested that local monoamine release could be evoked by stimulation, these studies were performed with clinically irrelevant stimulation parameters (Davidson and Stamford 1993; Trout and Kruk 1992; Kennedy et al. 1992). DBS of the NAc on monoamine release in distal regions has also received little attention. Two studies reported on the effect of NAc stimulation on mPFC monoamines in postmortem tissue. Sesia et al observed no alterations after acute stimulation, while Falowski et al demonstrated a decrease of catecholamine concentrations after chronic, continuous stimulation (Falowski et al. 2011; Sesia et al. 2010). We found increases in the release of dopamine and serotonin in the mPFC, and dopamine and noradrenaline in the OFC immediate after onset of stimulation in the NAc core. Interestingly, the rapid increase in prefrontal monoamine release 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 led to a simultaneous increase of monoamines in both the mPFC and the OFC (Denys et al. 2004a; Huang et al. 2006), although it should be noted that the drug-induced increases are clearly higher. The similarity in these direct effects on monoamine levels may indicate a possible common factor of these effective therapies for OCD.

Despite evidence of altered monoamine release following stimulation, it still is questionable whether these monoamine changes really are the underlying mechanism of the rapid effects of DBS in OCD. For example, in pharmacotherapy it is known that the therapeutic effects of SSRIs in OCD are usually not seen within 8 weeks of treatment while changes in monoamine levels following SRI intake occurs within hours. This time lag suggests that altered monoamine levels are not responsible for the therapeutic effects of SSRIs in OCD. El Mansari (El Mansari and Blier, 2006) have argued on the basis of their electrophysiological work that the time lag between SRI intake and clinical efficacy might be explained by a slower desensitization of the 5-HT$_{1A}$ auto-receptors in the OFC, a brain region specifically implicated in OCD. With DBS the rapid behavioural effects occur within minutes to hours and are therefore unlikely explained by a slow process like desensitization of the 5-HT$_{1A}$ autoreceptor.

However, the distal effect of DBS on the monoamines in the PFC does complement to the increasing amount of evidence from indirect and direct measurements of brain activity that DBS of the NAc modulates the dynamics of a broader network than just the NAc (McIntyre and Hahn 2010). Following 90 minutes of DBS in the NAc core in rats, alterations of local field
potential oscillations and evoked responses were observed not just in the NAc, but also in the mPFC, lateral OFC and the mediodorsal thalamus (McCracken and Grace2009). A study in morphine treated rats showed that DBS in the NAc suppressed the firing of neurons locally in the NAc but also distally in the ventral pallidum (Hu et al.2011). Neuroimaging studies have shown changes of various brain regions such as the PFC following acute DBS of the NAc and the ventral capsule/ventral striatum (Bewernick et al.2010;Knight et al.2013;Rauch et al.2006;van Laere et al.2006). A recent study of our group showed in OCD patients that DBS of the NAc normalized NAc activity, reduced excessive connectivity between the NAc and the PFC, and decreased low-frequency oscillations in the PFC (Figee et al.2013). Antidromic activation of corticostriatal fibers might be responsible for these effects of NAc stimulation on the PFC (Figee et al.2013;McCracken and Grace2007). Thus, DBS of the NAc appears to normalize the pathological activity of the neuronal network in the stimulated brain area as well as of distant brain areas.

The effect of DBS on anxiety and compulsivity
The data of this thesis clearly show effects of DBS on anxiety and compulsivity. First, stimulation of the IC exclusively reduced conditioned anxiety while stimulation of the CAU decreased both conditioned and unconditioned anxiety. This suggests that DBS may have a differential effect on unconditioned and conditioned anxiety depending on the stimulation area. There is currently limited preclinical research available of the role of the IC or the CAU on anxiety and compulsions. One preclinical study demonstrated that 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), while our study is the first to report on the impact of IC stimulation on anxiety. Rodriguez-Romaguera et al also reported that DBS of the CAU had distal effects on cell plasticity in the mPFC, OFC as well as the amygdala, three areas know to be involved in fear and anxiety (Rodriguez-Romaguera et al. 2012). They suggested that these alterations of the prefrontal-amygdala circuit could be due to the activation of corticofugal fiber bundles that run from the PFC through the striatum and the ventral capsule to the amygdala and the thalamus (Rodriguez-Romaguera et al. 2012). Most likely, DBS in the IC will modulate the same set of corticofugal fibers and therefore also the prefrontal-amygdala circuit. In concurrence with this, neuroimaging studies have shown activation of the PFC and the amygdala following DBS in the IC/ventral striatum (Abelson et al.2005;Knight et al.2013;Nuttin et al.2003;Rauch et al.2006;van Laere et al.2006). The changes in the prefrontal-amygdala circuit, induced by DBS could explain the anxiolytic effects in our study and are in line with the hypothesis that DBS modulates the dynamics of a broader network.

Second, DBS in the IC also affected compulsive behaviour. The sapap3 mutant mice initiated the grooming behaviour more often but aborted the individual grooming bouts quicker following DBS. It is, however, debatable whether these effects on compulsive grooming are a primary
effect of stimulation or are secondary to a possible anxiolytic effect of DBS in the IC. In OCD patients it is thought that the compulsive actions are performed to reduce the heightened levels of anxiety. In addition, DBS in OCD patients first causes a rapid drop in anxiety before a reduction in compulsivity is accomplished. It has been suggested that this initial reduction of anxiety is necessary for the compulsive behaviour to decline (Denys et al.2010). The sapap3 mutant mice show in addition to compulsive grooming behaviour also an increased anxiety level (Welch et al.2007). Data in this thesis showed that IC stimulation in rats can reduce conditioned anxiety. Possibly, this anxiolytic effect of DBS might be the underlying cause of the reduction in the compulsive grooming behaviour in the sapap3 mutant mice.

Despite an increasing amount of clinical and preclinical research, it is still open for discussion what the optimal brain target is for DBS. 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). Clinical experience suggests that DBS in OCD patients solely affects the symptom related conditioned anxiety that is the subject of obsessions and compulsion, and not the general, unconditioned anxiety. In our behavioural studies we found no effect of NAc or BNST stimulation on anxiety and compulsivity while CAU stimulation influenced both unconditioned and conditioned anxiety. The fact that IC stimulation in rodents uniquely affects conditioned anxiety and shortens the grooming bout combined with clinical data suggests that of the targets we tested, the IC might be the best candidate to be responsible for the efficacy of DBS.

8.4 Future research

Several questions follow the main outcomes of our experiments. The first question that emerges from this thesis is: What is the behavioural relevance of the monoamine changes in the PFC during stimulation of the NAc core? In our behavioural experiments there was no effect of NAc stimulation on anxiety or on compulsivity. This suggests that the monoamine increase in the PFC is not related to or is not sufficient enough to alter anxiety or compulsive behaviour. We did find an increase in locomotor activity after stimulation of the NAc core. It has been shown previously that simultaneous activation of prefrontal monoamines may be indicative of a generalized arousal effect (Feenstra2007;Marrocco et al.1994;Quinkert et al.2011). The increased monoamine release and locomotor activity may be connected to a generalized arousal effect. However, these interpretations have to be taken carefully because the behavioural experiments were not combined with microdialysis. In addition, our microdialysis studies were performed in freely moving animals that were not exposed to any form of external stimuli besides the access to food and water. Basal monoamine levels may be altered by placing the animals in behavioural paradigms, which could influence the effect
DBS has on monoamine levels. To confirm a possible link between the increased locomotor activity and the increased monoamine levels, our activity test needs to be combined with microdialysis.

Although stimulation in the NAc did not have behavioural effects, there were strong effects of IC stimulation in our behavioural experiments. Stimulation of corticofugal fibers modulates distal brain areas. These changes in distal brain areas and possibly the prefrontal-amygdala circuit could underpin the reported behavioural effects. In line with this, neuroimaging studies have shown alterations in activity in several brain areas such as the PFC, striatum and the amygdala following DBS in the IC/ventral striatum (Abelson et al.2005;Knight et al.2013;Nuttin et al.2003;Rauch et al.2006;van Laere et al.2006). However, the effects of IC stimulation on neurochemical processes such as neurotransmitter levels are unknown. The second question therefore would be: What is the effect of IC stimulation on neurochemical processes locally or in the distal brain areas suggested by the neuroimaging studies? There is clear evidence that, besides the monoamines, GABA and glutamate are linked to anxiety and compulsions as well (Pittenger et al.2011;Riaza Bermudo-Soriano et al.2012;Shephard1987;Wu et al.2012). Possibly GABA and glutamate play a more important role than the monoamines in the rapid behavioural effects of DBS. Future research should therefore also focus on the effect of DBS on these fast-acting neurotransmitters.

The third question is: Is the reduction in compulsivity a primary effect of stimulation or secondary to the anxiolytic effects of DBS? It has been suggested that the initial reduction of anxiety following the onset of DBS is necessary for the compulsive behaviour to decline (Denys et al.2010). The sapap3 mutant mouse is a suitable OCD animal model to investigate this question as it exhibits besides the compulsive grooming behaviour also an increased anxiety-like behaviour (Welch et al.2007). It is however not an easy question to answer and multiple initial steps are necessary to start approaching it. First, is the increased anxiety-like behaviour correlated with the compulsive grooming behaviour in the sapap3 mutant mice. In other words, are high levels of anxiety predictive of compulsive grooming. Second, does IC stimulation reduce conditioned anxiety in the sapap3 mutant mice as we found in rats. Third, if the IC stimulation has an anxiolytic effect is the extent of the reduction correlated with the stimulation induced alteration of the grooming bouts.

Fourth, 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 modify neurotransmitter release in the PFC? The NAc proper consists for the most part out of grey matter. However, white matter fascicules are running through the NAc, probably representing corticofugal fiber bundles, as described for primates (Lehman
et al.2011). It is presently not known whether stimulation in rats mainly activates incoming, outgoing or passing fiber bundles, which makes it difficult to determine how DBS in the NAc modifies the PFC. Three possible mechanisms could explain the increase in prefrontal monoamine release following NAc stimulation: Increases in the monoamine release in the PFC could result from (1) stimulation of accumbens efferents or passing fibers resulting in increased firing rates of the monoaminergic neurons in the midbrain and the pons which project to the PFC, from (2) presynaptic activation of monoaminergic prefrontal terminals through modulation of the CSTC loop (Schicker et al.2008) or from (3) antidromic activation of corticostrial fibers (McCracken and Grace2007). It would be interesting to follow up our microdialysis experiment by investigating if one of these three options is responsible for the effect we found.

The fifth question relates to the behavioural effects we found after IC stimulation. As it seems, the common factor underlying the behavioural effects in animals and the clinical data may be the stimulation of the previously mentioned corticofugal tracts. It is however unknown which of the corticofugal tracts is responsible for the efficacy of DBS. In future research it would therefore be interesting to answer the following question: Which fiber bundles are modulated to attain the significant decrease of anxiety and compulsivity with DBS? Optogenetics is an excellent technique to selectively modulate the different corticofugal fiber bundles. However, we first need to know their exact organization and target areas. Anatomical tracing studies investigating these corticofugal fibers by virus injections in the prefrontal cortex of the rat will be the first step. This will show us the location of the expression of the virus in the fiber bundles itself and in possible target areas. Subsequently investigating the behavioural effects of optical modulation of these target areas will provide information on the specific fiber bundles mediating the behavioural effects of DBS.

The last question regards all the studies we have done. Studies into the effect of DBS in animals, as in our microdialysis, neurogenesis and behavioural studies, typically use short stimulation periods (minutes to hours) while in patients the stimulation is chronic. An electrophysiology study demonstrated that extending the stimulation period of DBS in the NAc from 90 minutes to 5 days can cause significant changes. The initial changes in local field potential oscillations and evoked responses were replaced by altered coherence between different brain regions (Ewing and Grace2012). A logical question therefore is: What will be the effect of longer stimulation periods on neurochemical and behavioural measurements?
8.5 Conclusion

In conclusion, we see a strong impact of DBS on several aspects of OCD. DBS in different brain areas affects compulsive behaviour, conditioned and unconditioned anxiety. DBS in the IC shows the most promising behavioural results by uniquely reducing conditioned anxiety and by shortening the compulsive grooming bout in the sapap3 mutant mouse. This suggests that the IC is possibly the best target for DBS in relation to OCD. Further research into the stimulation of the IC has to clarify which changes in cellular or system level are responsible for these behavioural effects. The behavioural relevance of the effect of DBS in the NAc core on the monoamines in the PFC is as of yet unclear. Further behavioural experiments combined with microdialysis have to be performed to investigate a possible link between the increase in PFC monoamines and locomotor behaviour during DBS in the NAc core. DBS is widely used in clinical settings for several different diseases. However, the lack of knowledge of the optimal target for DBS, the neurobiological mechanisms underlying the rapid effects of DBS and the specific impact of DBS on the different symptoms of the diseases prevents the full potential of its application. This thesis could be viewed as one of the building blocks to achieve this.
References


Chapter 8


