Understanding deep brain stimulation in obsessive compulsive disorder: A preclinical study into the mechanism of action and behaviour
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Chapter 5

Acute stimulation of the nucleus accumbens has no effect on hippocampal neurogenesis

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Short communication
Treatment refractory patients with obsessive compulsive disorder (OCD) or major depressive disorder (MDD) may be treated effectively with deep brain stimulation (DBS) targeted at the nucleus accumbens (NAc) (Denys et al. 2010; Huff et al. 2010; Malone, Jr. et al. 2009; Schlaepfer et al. 2008). However, little is known about the neurobiological mechanisms underlying the effects of DBS. Recent studies showed that DBS in the anterior nucleus of the thalamus, entorhinal cortex and the subthalamic nucleus can increase the neurogenesis in the dentate gyrus (DG) in rodents (Encinas et al. 2011; Khaindrava et al. 2011; Stone et al. 2011; Toda et al. 2008). This led us to start a pilot study to investigate the effect of DBS in the NAc on neurogenesis in the DG.

In this study we applied bilateral bipolar DBS (300µA, 120Hz, biphasic, pulse width 80 µs) in the NAc (A +1.5mm, L ±0.9mm, V 4.8mm) in adult male C57Bl6 mice in combination with 5-bromo-2-deoxyuridine (BrdU) injections. The experiment was divided into a one and a five day experiment to investigate acute and intermediate effects of stimulation. In the one day experiment mice received an injection of BrdU (150 mg/kg i.p. in saline) after one hour of (sham) stimulation after which the stimulation continued for another three hours. Two hours after the end of the stimulation mice were deeply anesthetized with pentobarbital and perfused with 0.9% saline followed by 4% paraformaldehyde in a phosphate buffered saline. In the five day experiment mice were also (sham) stimulated on day 1 for 4 hours and received a BrdU (150 mg/kg i.p) injection after 1 hour of stimulation. Subsequently, the animals also received a BrdU injection on day 3, 4 and 5 at approximately the same time as on day 1. Due to practical issues, there were different injection schemes. One control animal received no injections on day 1 but on day 2, 3, 4 and 5 while two stimulated animals received only injections on day 3, 4 and 5. On the fifth day the animals were perfused with 4% paraformaldehyde two hours after the last BrdU injection. In both experiments, the head of the animal including the electrodes were post-fixed for two days at 4°C. Brains were then removed and post-fixed overnight prior to immersion in 20% sucrose at 4°C. Finally, brains were directly frozen on dry ice and stored at –80°C before being sectioned using a cryostat. Ten μm coronal sections of the NAc were stained with Cresyl violet for determination of the exact location of the two stimulation electrodes. For immunohistochemistry, the hippocampal area was collected from AP -1.3 till -2.5. The procedure for the immunohistochemical staining is extensively described by Kamphuis et al (Kamphuis et al. 2012). For each mouse, in approximately 10 sections, we counted the total number of cells that incorporated BrdU (ipsilateral and contralateral combined) and the mean of the number of BrdU positive cells per DG was calculated for each animal.

In the one day experiment the two mice of the sham stimulated group had a mean of 10.4 and 10.0 BrdU positive cells per DG and the NAc stimulated group (3 mice) a mean of 9.2 ± 1.2 BrdU positive cells, while in the five day experiment the sham stimulated group (3 mice) had a
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mean of 26.4 ± 4.3 BrdU positive cells and the NAc stimulated group (3 mice) a mean of 18.2 ± 2.0 BrdU positive cells in the DG (fig. 1). There was no significant difference (unpaired student t-test) between the sham stimulated group and the NAc stimulated group for either the one day or five day experiment.

DBS NAc has no effect on neurogenesis in the DG

Examples of the BrdU-immunostaining patterns in the DG are shown in Fig 2, illustrating the accumulation of BrdU-labeled nuclei in the subgranular zone.

This pilot study is the first to report on the effect of DBS in the NAc on neurogenesis in the subgranular zone of the fascia dentate in the hippocampus. Using freely moving animals, our preliminary results suggest that stimulation for 4 hours in the NAc has no effect on neurogenesis in the DG on the same day or 5 days after stimulation. The non-significant visual difference between the sham stimulated and stimulated group in the 5- day experiment may be explained by the fact that 2 of the 3 stimulated animals only received 3 BrdU injections compared to the 4 BrdU injections for the other animals.

Our results contrast with other studies showing profound effects of high frequency stimulation on neurogenesis in the DG. Toda et al found a 2- to 3- fold increase in BrdU-positive cells in the DG with stimulation of the anterior nucleus of the thalamus for 1 hour (Toda et al.2008). This was followed up by Encinas et al who showed that these BrdU-positive cells are amplifying

Fig 1: Effect of acute stimulation of the NAc on neurogenesis (mean number of cells per section ± SEM) in the DG (ipsi and contralateral combined) on day 1 or day 5 after stimulation.
neural progenitors (ANP) that may differentiate into new neurons in the DG (Encinas et al.2011). Khaindrava showed that stimulation of the STN in 6-hydroxy dopamine lesioned rats reversed the lesion-induced decrease in cell survival in the subventricular zone-olfactory bulb continuum, striatum and the DG (Khaindrava et al.2011). Furthermore, a 1.5 to 2-fold increase in adult neurogenesis in the DG was induced by entorhinal cortex stimulation (Stone et al.2011).

Our study differs in several aspects from the studies mentioned above. First, in contrast with the anterior nucleus of the thalamus and the entorhinal cortex, neither the NAc nor the STN have direct projections to the DG (Shibata 1993; Stone et al.2011). The STN stimulation, performed in 6-hydroxy dopamine lesioned rats, did not affect neurogenesis but was only successful in reversing the lesion-induced decrease in cell survival in an animal model of Parkinson disease (Khaindrava et al.2011). This finding combined with the absence of direct projections between the STN and DG suggests that their results were found due to an effect of STN stimulation on the lesion and not by influencing the DG directly. It is unclear what the effect of STN stimulation is on neurogenesis in healthy animals. Second, there is a difference in species used. Mice, rats and the 6-hydroxy dopamine animal model for Parkinson differ in brain morphology, which may have influenced neurogenesis (Amrein et al.2004; Kempermann and Gage 2002). Third, though contradictory effects have been reported, anesthesia may have an effect on neurogenesis (Erasso et al.2012; Tung et al.2008). In three of the DBS studies mentioned above the rodents were under anesthesia while being stimulated, while our study and the study by Khaindrava et al was performed in freely moving animals. Lastly, different stimulation periods (1 hour till 8 days), electrodes (e.g. monopolar versus bipolar, material) or stimulation parameters were used. It is unclear what the influence of the mentioned variations

![Fig 2: BrdU staining in the subgranular zone of the fascia dentate in the hippocampus. Dapi (blue) was used as a nuclear counterstain. Picture (A) shows a NAc stimulated mouse while picture (B) shows a sham stimulated mouse from the one day experiment. Blue = DAPI, red = BrdU](image_url)
is on neurogenesis and if it can explain the differences of the effect of stimulation. In addition, although this pilot study has a limited amount of animals per group, the power to detect a 2-fold increase in neurogenesis, as reported in the other studies, was adequate in this study (1 day experiment, $\beta = 0.998$, 5 day experiment, $\beta = 0.993$).

To conclude, we found no evidence for a clear effect of acute NAc stimulation on neurogenesis in the DG which suggests that hippocampal neurogenesis does not underlie the therapeutic effect of DBS in the NAc. 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. Though DBS is used in clinical settings, the mechanism of action of DBS is still largely unknown. Further knowledge of the direct and indirect processes in the regions receiving stimulation as well as those connected and in close vicinity is necessary.
Chapter 5

References


