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Authors: G. Bazzu, P.A. Serra, R. Hamelink, M.G.P. Feenstra, I. Willuhn, D. Denys

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Monitoring Deep Brain Stimulation by measuring regional brain oxygen responses in freely moving mice

Bazzu G.[1], Serra P.A.[1], Hamelink R.[2,3], Feenstra M.G.P.[2,3,*#], Willuhn I.[2,3,*], Denys D.[2,3,*]

[1] - Department of Medical, Surgical and Experimental Sciences, Section of Pharmacology,, University of Sassari, Sassari, Italy.

[2] - Netherlands Institute for Neuroscience, an institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam;

[3] - Department of Psychiatry, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands.

* contributed equally

correspondence: Matthijs G.P. Feenstra Ph.D., Netherlands Institute for Neuroscience, Meibergdreef 47, 1105 BA, Amsterdam, The Netherlands.

Emal: m.feenstra@nin.knaw.nl

Highlights

- We combined deep brain stimulation with amperometric measurements of oxygen in the mouse brain.
- The effects of behavioural stimuli like novelty were easily detectable.
- DBS was shown to reduce the effects of novelty in the striatum.
- The method allows chronic studies of DBS effects on brain activity in freely-moving mice.

Abstract

Background

Translational studies investigating the effects of deep brain stimulation (DBS) on brain function up to now mainly relied on BOLD responses measured with fMRI. However, fMRI studies in rodents face technical and practical limitations (e.g., immobilization, sedation or anesthesia, spatial and temporal resolution of data). Direct measurement of oxygen concentration in the brain using electrochemical sensors is a promising alternative to the use of fMRI. Here, we tested for the first time if such measurements can be combined with DBS.

New Method

We combined bilateral DBS in the internal capsule (IC-DBS) with simultaneous amperometric measurements of oxygen in the medial prefrontal cortex (prelimbic area) and striatum of freely moving mice. Using a two-day within-animal experimental design, we tested the effects of DBS on baseline oxygen concentrations, and on novelty- and restraint-induced increases in oxygen concentration.

Results

Basal oxygen levels were stable across the daily sampling periods. Exposure to novelty and immobilization reproducibly increased oxygen concentrations in both areas. IC-DBS did not significantly alter basal oxygen, but reduced the novelty-induced increase in the striatum.

Comparison with Existing Method(s)

Amperometric detection of brain oxygen concentration with high temporal and spatial resolution can be performed in a number of key brain areas to study the effects of DBS in animal models of disease. The method is easily implemented and does not require expensive equipment or complicated data analysis processes.

Conclusions

Direct and simultaneous measurement of brain oxygen concentration in multiple brain areas can be used to study the effects of bilateral DBS neuromodulation on brain activity in freely moving mice.

Keywords: brain oxygen; in vivo electrochemistry; deep brain stimulation; internal capsule;

corticostriatal.

1. Introduction

Deep brain stimulation (DBS) is widely used for treatment-refractory neurological and psychiatric disorders (Wichman & DeLong, 2006; Lozano & Lipsman, 2013; Denys et al, 2012; Okun, 2014). However, despite considerable advances, our understanding of the DBS mechanism of action remains limited. Although DBS effects are presumably manifold and widespread, a normalization of disease-related aberrant activity in only a subset of neuronal circuits is believed to underlie therapeutic effects (Bourne et al, 2012; Figee et al, 2013; Le Jeune et al, 2010). Such effects can be visualized by fMRI-based measurement of brain activity, as often applied in clinical studies. Similar studies can be performed in experimental animals and would therefore be the method of choice for translational approaches. However, fMRI is an expensive technique which requires high financial and personal investments and complex data analysis. Moreover, the limitations of fMRI studies in small rodents are considerable and include the need for strict immobilization, the use of anesthesia or sedation, and the temporal and spatial resolution, which is even more restricted due to smaller brain size (Hoyer et al, 2014). Together, these factors limit the value of rodent fMRI studies for translational applications. An alternative would be to assess the same parameter as used in fMRI (i.e., changes in brain regional cerebral blood flow) in a different way. Amperometric measurements of brain oxygen fluctuations (Bolger & Lowry, 2005; Serra et al, 2010) provide an index of regional cerebral blood flow and can serve as a surrogate for fMRI BOLD responses (Lowry et al, 1997; 2010; Francois et al, 2012), yet avoid the above-mentioned limitations and offer greater temporal resolution than fMRI. Measurements using one or more amperometric sensors have been reported in freely moving rats and mice in one or more brain areas (Li et al, 2015; McHugh et al, 2014) and may enable the assessment of DBS effects on neuronal circuits by monitoring oxygen fluctuations in key brain areas. Up to now, the possibility of such a combination has not been explored.

The aim of this study was to test if we could implement continuous measurements of oxygen concentrations in two different brain areas in awake and freely moving mice to study the effects of bilateral DBS. We used a two-day counterbalanced within-animal study design (DBS-on and DBS-off) that included measurements of oxygen levels under basal conditions and during behavioral challenges that were expected to increase these levels. DBS was applied to the internal capsule (IC), which is an effective target in the treatment of therapy–refractory psychiatric disorders, including obsessive-compulsive disorder (OCD) (Alonso et al, 2015). We recently also reported anxiolytic activity of IC-DBS in normal rats (van Dijk et al, 2013) and anticompulsive effects of IC-DBS in a mouse model of OCD (Pinhal et al, 2018).

Our results indicate that this approach is feasible, reliable, and promising overall.

2. Material and Methods

2.1 Reagents and Solutions

All chemicals were analytical reagent grade, used as supplied, and dissolved in distilled, deionized water (MilliQ). Graphite powder (Fluka, ref 78391) and collodion solution (4% cellulose nitrate in ethanol/diethyl ether from Fluka, ref 09986) were purchased from Sigma-Aldrich (Milan, Italy). Phosphate buffer saline (PBS) solution was made using NaCl (137 mM), KCl (2.7 mM), Na_2HPO_4 (8.1 mM), and KH_2PO_4 (1.47 mM) (all from Sigma), and adjusted to pH 7.4. Nitrogen-purged and oxygen-saturated solutions, used for *in vitro* calibrations at low concentrations, were prepared by dissolving (bubbling) ultrapure (>99.9%) oxygen and nitrogen gases in 10 mL of PBS for 1 h. The air-saturated solution of PBS (21% oxygen) was prepared by bubbling filtered air in 10 mL of PBS for 1 h, using a diaphragm air pump (DAP). All *in vitro* calibrations were performed in freshly prepared N_2 and O_2 solutions under standard conditions of pressure and temperature.

2.2 Animals

Male C57BL/6 mice (Harlan, the Netherlands), weighing 28-40 g, were used for *in vivo* experiments. Mice were maintained under a reversed day-night cycle (12 h/12 h dark/light cycle, light at 19.00 h; room temperature 21 °C), with food and water *ad libitum*.

We present the data of four mice with verified DBS and amperometric electrode placements. Data from another four mice were not used because of off-target amperometric (1) or DBS (3) electrode placements.

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.

2.3 Construction and Calibration of Oxygen Microsensors

The oxygen microsensors were made by modifying a procedure described previously (Bazzu et al., 2009). Approximately 1 mm of Teflon-insulated silver wire (30 mm in length; Ø 125 μm, Advent Research Materials, Suffolk, U.K.) was exposed and inserted into a silica capillary tube (5 mm in length; Ø 180 μm, Polymicro Technologies, Phoenix, AZ) and then partially filled with graphite-loaded (55% w/w) epoxy resin (Araldite-M, Sigma-Aldrich, Milan, Italy) attained by mixing 850 mg of graphite with 500 mg of Araldite-M and 200 mg of hardener (Migheli et al., 2008). Electrical contact was guaranteed by silver wire. After 1 h at 100 °C, the surface of the working electrode was polished using sandpaper (1200 grain). After treatment with cellulose nitrate the electrodes were dried. The final oxygen microsensors were carbon-composite disk electrodes having a diameter $\approx$ 180 μm and an active surface $\approx 2.5 \times 10^{-2}$ mm^2.

Oxygen microsensors were characterized and calibrated using a three-electrode system (working, WE; reference, Ref and auxiliary, Aux, electrodes), a four-channel potentiostat (eDAQ QuadStat, e-Corder 410, eDAQ, Denistone East, Australia) and exposing electrodes to variable oxygen concentrations by means of an airtight electrochemical cell. All electrodes were briefly immersed in 10 mL of PBS in a glass beaker tightly closed with a modified cap. Two holes in the cap allowed

modification of gas saturation by bubbling ultrapure (>99.9%) nitrogen in 10 mL of PBS for 1 h or adding known amount of oxygen-saturated PBS solution by means of an Hamilton syringe. Oxygen reduction was experimentally demonstrated on microsensors surface at -700 mV vs Ag/ AgCl using cyclic voltammetry (CV) with a scan rate of 25 mV/s.

Constant potential amperometry (CPA) was used for all *in vitro* calibrations and *in vivo* experiments, fixing the oxygen reduction potential at -700 mV vs Ag/AgCl reference electrode. As previously demonstrated, no significant interference signals were observed when the sensors were exposed to other electroactive molecules present in the cerebral extracellular fluid (such as ascorbic acid (AA); uric acid (UA); dopamine (DA); 3,4-dihydroxy-phenylacetic acid (DOPAC); homovanillic acid (HVA) (Migheli et al., 2008; Bazzu et al., 2009). Oxygen sensor calibrations were performed after having immersed the sensors in the above-mentioned airtight electrochemical cell with fresh N_2-purged PBS (N_2 100%) and then, after the baseline current was stabilized, a five point calibration was performed at low oxygen concentrations (from 0 to 125 μM) by adding different aliquots (+200, +204, +208, +212, and +216 μL) of an oxygen-saturated solution (1.25 mM, oxygen 100%), obtained as described in reagent and solution paragraph. Sensors with an oxygen detection limit ≤5 μM and an oxygen sensitivity higher than 200 pA $μM^{-1}$ were selected for *in vivo* implantation. The Ag/AgCl reference (ref) electrode, was prepared by immersing 1 mm silver wire (Ø = 125 μm) in a saturated KCl solution and applying +500 mV for 1 min.

2.4 DBS electrodes construction

The DBS bipolar electrodes consisted of two twisted teflon coated platinum/iridium wires (30 mm in length; Ø = 70 μm) (van Dijk et al, 2011). The tips of the wires, with one pole 500 μm ventral to the other, were cut off straight which formed an exposed surface area per pole of 3.85×10^{-3} mm^2. Mice were sham-stimulated or stimulated using a waveform generator (DS8000, World Precision Instruments, Sarasota, FL, USA) connected through a stimulation isolator (DLS100, World Precision Instruments, Sarasota, FL, USA), using the following parameters: Period: 8.32 ms (+/- 120Hz), Pulse

Width: 0.08 ms, Waveform: Bipolar pulse, Amplitude: 3 V (via Isolator set on 1 mA gives 300 µA). These settings have been shown to be both safe and functionally effective, as no tissue damage was observed in post-mortem brain sections and specific neurochemical and behavioral effects were reported in rats (increased efflux of monoamines in the medial PFC and reduction of conditioned anxiety, van Dijk et al, 2012, 2013) and in mice (reduced compulsive grooming, Pinhal et al, 2018). Regarding safety issues of the present settings, we also refer to the discussion of DBS settings in van Dijk et al (2011). Sham-stimulation was carried as described above, whereby the stimulation isolators were turned off.

2.5 Stereotaxic Surgery

Stereotaxic surgery was performed under isoflurane (1-3% in 1:1 oxygen-air) anesthesia; subcutaneous preoperative meloxicam analgesia (1 mg/kg) and eye ointment were administered. Body temperature during anesthesia was kept constant at 36.5 °C using a rectal thermometer connected to a heating pad. A small incision was made to expose the skull of the animals placed in a Kopf stereotactic apparatus (Kopf Instruments, Tujunga, CA), and lidocaine was used for local anesthesia. Oxygen microsensors were implanted in both the right prelimbic cortex (PL) and striatum, and DBS electrodes were bilaterally implanted in the internal capsule (IC) using the following coordinates (Franklin & Paxinos, 2007) (AP and ML from bregma and DV from dura): **PL**: A/P= +1.54 and M/L =-0.25, and D/V = -2.5; **Striatum**: A/P = +0.5 and M/L =-1.5, and D/V = -3.5. **IC**: A/P = -0.58 and M/L = ±1.4, and D/V = -4.3. The reference and auxiliary electrodes were implanted in the left parietal cortex.

Oxygen sensors, reference, auxiliary, and DBS electrodes were connected to a 8-pin DIP IC socket (Figure 1A) and then fixed to the skull surface with 3 stainless steel screws and dental acrylic cement (Figure 1B). The final weight of the head implant was about 1.7 ± 0.2 grams. After surgery, mice were injected with saline (200 µL) subcutaneously to prevent dehydration. Animals were allowed to recover first in a couveuse (36.5 °C) (Figure 1C) until they were awake and then housed in individual

cages for at least one week before further experimentation. The tube method (Hurst & West, 2010) was used for daily handling during 8 days and this plastic tube remained in the home cage.
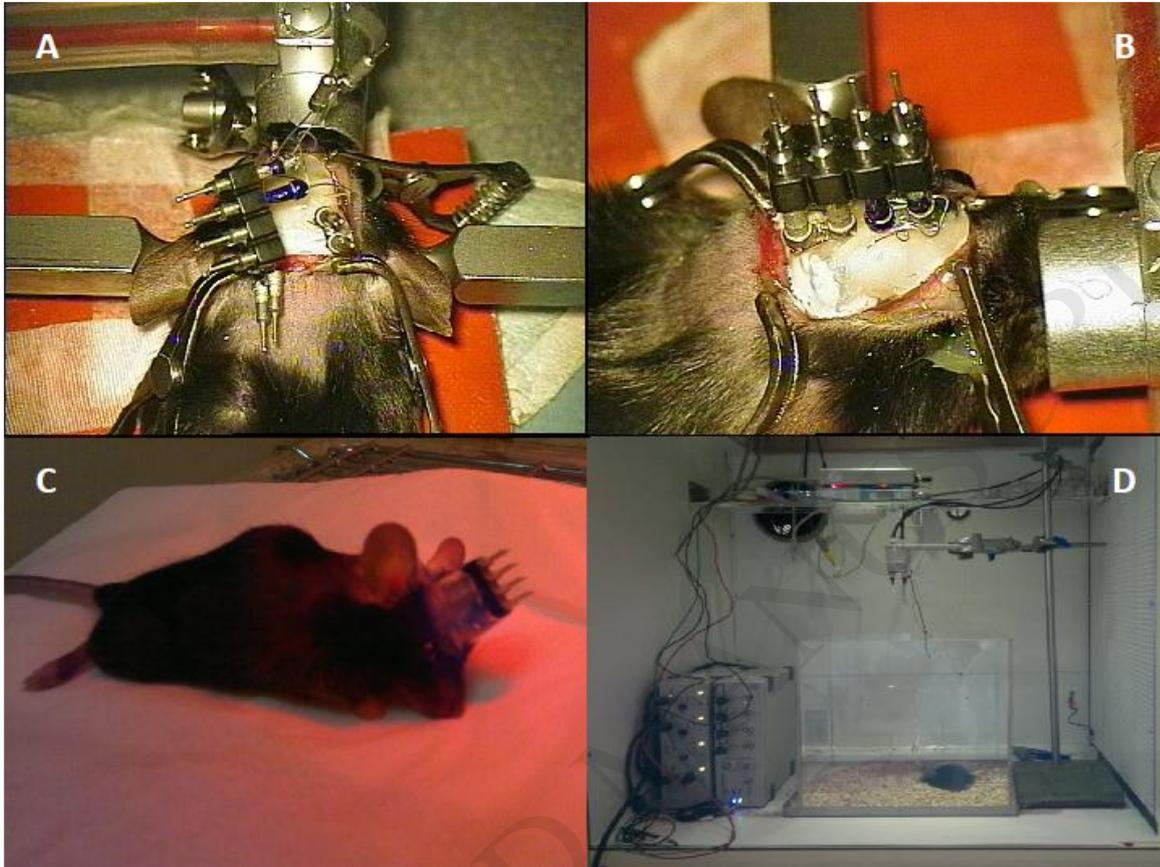

Figure 1. *Surgery procedures and experimental set-up.*

Stereotaxic surgery was performed under Isoflurane anesthesia. Oxygen sensors, reference, auxiliary, and DBS electrodes were connected to a 8-pin DIP IC socket (A) and fixed to the skull surface with screws and dental acrylic cement (B). After surgery, animals were allowed to recover in a couveuse (36.5 °C) until awake (C). During experiments, mice were placed in a 25 cm x 25 cm x 25 cm plastic chamber. Via a commutator, oxygen sensors and DBS electrodes were connected to the potentiostat and stimulation equipment, respectively, allowing unrestricted movement (D).

2.6 *In Vivo* Experiments

Seven days after surgery, mice were placed in a 25 cm x 25 cm x 25 cm plastic chamber using a plastic tube. Oxygen sensors and DBS electrodes were connected to eDAQ and DBS equipment, respectively. A commutator was used to allow unrestricted movement (Figure 1D).

We used a two-day, within-animal counterbalanced study design in which mice were tested for the effects of either sham-stimulation or DBS. As illustrated in Figure 2, the experiments were subdivided into four periods: a stabilization period, two baseline recording periods, and a behavioral test period. Briefly, after connecting the oxygen sensors and DBS electrodes, mice were placed into the test chamber and oxygen measurements began. After a 60 min. stabilization period brain oxygen was registered for two hours, starting with a baseline period (60
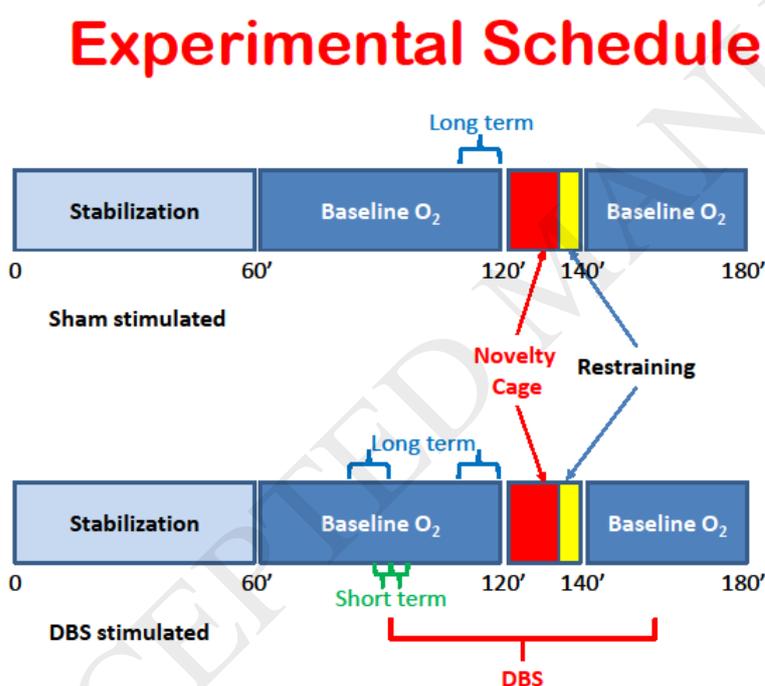

Figure 2. *Schematic representation of the two-day experimental schedule.*

*After the initial stabilization period, oxygen baseline currents (O$_2$) were registered daily for one hour immediately before and after behavioral testing (novelty and restraining), one day with DBS and one day with sham stimulation. The order of testing (sham or DBS) was counterbalanced across mice. 10-minute periods of raw data recording (**Long term**) were used as oxygen baseline. The DBS early effect on oxygen baseline was evaluated by comparing two minutes of raw data recording before and the two minutes after DBS initiation*

*(**Short term**). DBS was turned on 30 minutes before novelty exposure and turned off 20 minutes after restraining.*

min). Then, behavioral arousal and stress were induced by exposing animals to environmental novelty for 15 min followed by 5 min of manual restraining stress. For novelty exposure, animals were picked up using the plastic tube and placed into a neighboring novel cage of similar proportions containing bedding material, a piece of wood, and a plastic toy. Restraint stress was induced by taking the mice out of the novel cage, placing them on a metal grid, and immobilizing them by holding their scruff firmly for 5 min. After that, mice were returned to the experimental chamber and oxygen measurements continued for 40 min. Automated video registrations of behavioral activity were not possible because of the tethering device.

These experiments were carried out both in absence and in presence of DBS. On DBS days, isolators were turned on and the stimulation started 30 minutes before novelty exposure and turned off 20 minutes after restraining. DBS effects on oxygen concentrations were assessed both as short-term and long-term changes. Short-term effects were recorded in two subsequent 2-min periods (each subdivided in three 40-s periods), with DBS being turned on after the first two min. Long-term effects were determined in two 10-min periods (subdivided in three 3-min periods), one directly before DBS-ON, the other 20 min later, directly before the behavioral tests.

2.7 Histology

Location of oxygen microsensors and DBS electrodes were assessed by post-mortem histology. Following the experiments, mice were deeply anesthetized (pentobarbital 100 mg/kg), transcardially perfused (paraformaldehyde, PAF, 4 % in 0.1M PBS), and brains were removed. Brains were then post-fixed for 24 h in PAF and submerged in 30% sucrose in 0.1 M PBS for approximately 48h, then snap-frozen at -80ºC. Cresyl violet staining was carried out on 30-μm coronal brain sections, cut on a cryostat, and brain sections were examined under a microscope.

2.8 Statistical Analysis

Oxygen reduction currents are expressed in nanoamperes (nA) and given as baseline-subtracted (Delta - I) raw data. To improve the readability of the plotted data, the sign of the oxygen current is inverted. Oxygen concentrations are expressed in micromolar (µmol/L) and were calculated using an *in vitro* calibration factor for each sensor. Changes in oxygen concentrations after behavioral stimuli or after DBS onset are presented as percentage of preceding baseline concentrations.

To evaluate changes in oxygen levels during behavioral tests and the DBS effect, the mean of 6000 consecutive recorded sampling points (10 min) ± standard error of the mean (SEM) were used to compare the oxygen baseline values among experimental groups, while the mean of 1200 consecutive recorded points ± SEM, recorded during the maximum magnitude of oxygen changes, were used to compare behavioral tests effect both in absence and presence of deep brain stimulation.

The graphs provide both the individual data and the average values for the 3-4 mice. While statistical analysis was performed, the results should be regarded as provisional, given the small group sizes. We used a repeated-measures analysis of variance (ANOVA), with the Greenhouse-Geisser correction of the degrees of freedom for non-sphericity. When multiple contrasts were analyzed, the Sidak correction was used to adjust p values. A p-value ≤ 0.05 was considered statistically significant.

3. Results

3.1 Microsensor Response to oxygen in vitro

Oxygen microsensors were calibrated *in vitro,* 24 h after manufacturing, on the day of *in vivo* implantation. All *in vitro* calibrations were carried out in quiescent conditions using a 10-mL modified glass beaker, as described in the Materials and Methods section, connecting the microsensors to eDAQ potentiostat and a constant potential of -700 mV vs Ag/AgCl was applied.
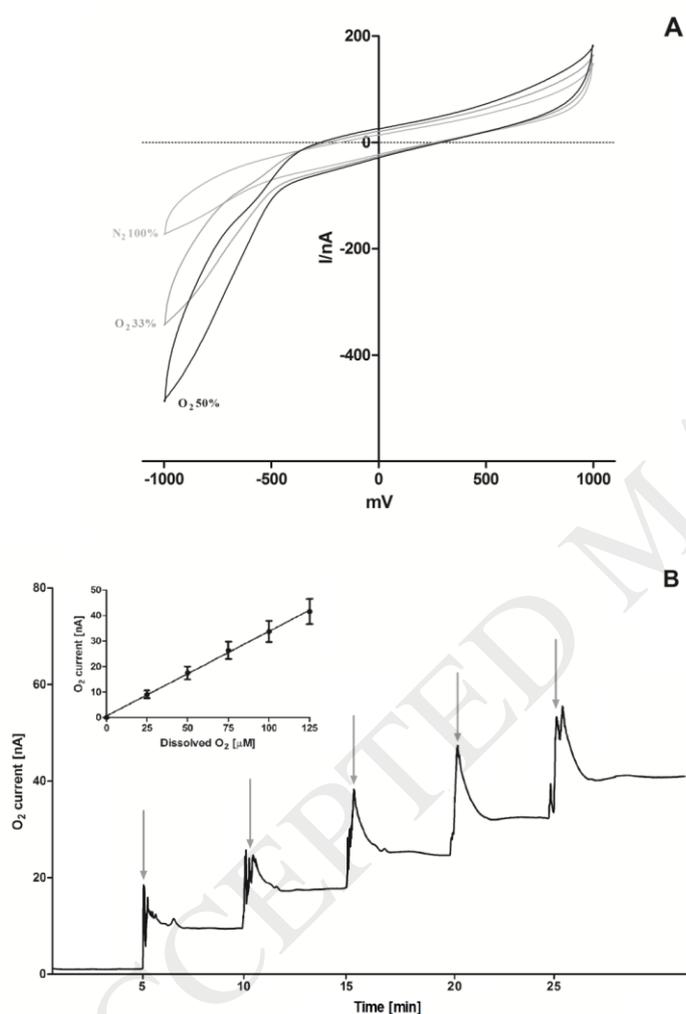

Figure 3. *Microsensor response to oxygen.*

Oxygen reduction on the microsensors surface, starting at -700 mV vs Ag/ AgCl, was demonstrated by cyclic voltammetry with a scan rate of 25 mV/s (A). Microsensor response to low oxygen concentrations was studied by constant potential amperometry (B). A five-point calibration (from 0 up to 125 μM) was performed adding known volumes (+200, +204, +208, +212, and +216 μL, arrows) of a saturated oxygen solution (1.25 mM)

to 10 mL of nitrogen-saturated PBS. All microsensors showed linearity (R^2 = 0.999; n = 24) and good sensitivity (slope of 365 ± 0.1 pA·µM⁻¹).

Oxygen microsensors were first exposed to N_2-purged PBS and then, when stability of background current was reached (1.6 ± 0.3 nA), several aliquots of an oxygen-saturated solution were added to the cell (Figure 3). After each oxygen addition, the solution was quickly mixed by means of a small magnetic bar (length = 10mm, Ø = 0.5 mm) while the oxygen reduction current was recorded, after stirring, under quiescent conditions. As illustrated in Figure 3 (inset), all microsensors showed excellent linearity (R^2 = 0.999; n = 24) and good sensitivity (slope of 365 ± 0.1 pA·µM⁻¹) comparable to previous published data (Bazzu et al., 2009) and a response time of <1 second.

3.2 Effect of DBS on baseline oxygen levels in prelimbic and striatal brain areas of freely moving mice

In absence of DBS, the registered oxygen baseline current on the first day of measurement was 9.94 ± 0.54 nA in the PL (n=4) and 8.66 ± 2.85 nA in the Striatum (n=3), respectively. The oxygen concentration was estimated using microsensor's averaged background current in N_2-purged PBS (1.6 ± 0.3 nA) and *in vitro* calibration, and it was found to correspond to 31.62 ± 8.02 µmol/L in the PL and 27.77 ± 8.35 µmol/L in the striatum. Baseline measurements were generally stable over two days of measurement (e.g. PL baseline on day 2 was 87-96% of day 1 for 3 animals), with one exception of a mouse (indicated in the figures by the star) with baseline concentrations 2-3-fold increased on the second measurement day.
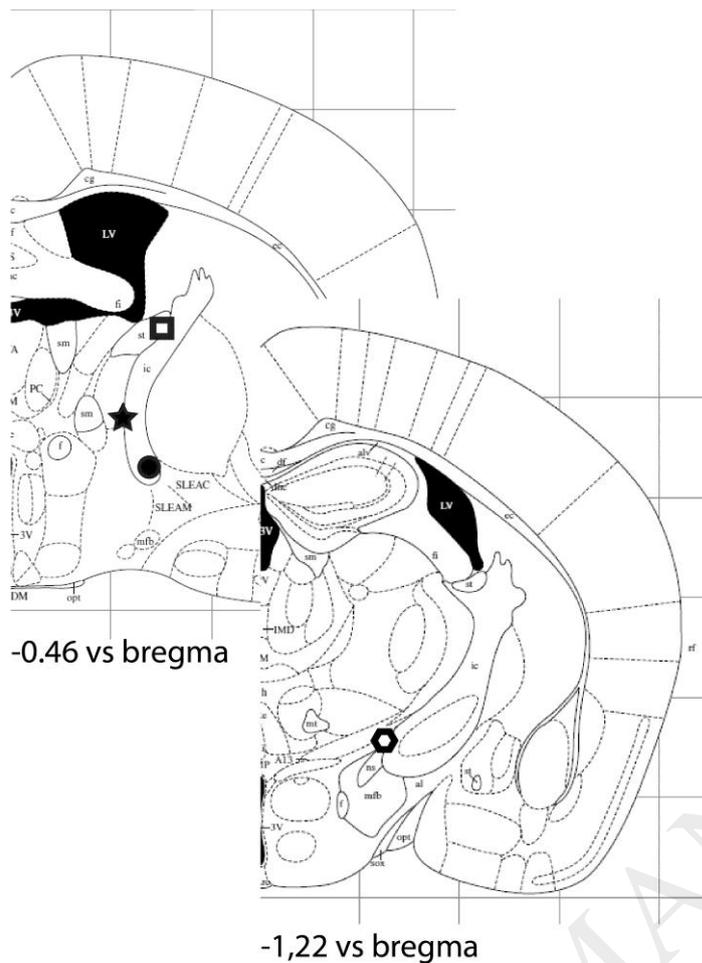
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Figure 4. *Placement of DBS electrodes.*

Shown are the positions of the electrode tips targeting the internal capsule in the right hemisphere.

The symbols represent the individual animals and correspond to the symbols used in the other graphs.

Figure 4 shows the position of the DBS electrodes targeting the IC of the right hemisphere, the hemisphere where amperometric recordings were carried out. Electrodes on the left hemisphere were in or next to the IC. The time course of the immediate effects of turning on DBS on normalized oxygen levels in the short term (6 consecutive periods of 40 s each) is presented in Figure 5A. The immediate responses were variable and the average small increases to 104% (PL) and 108% (striatum) were not significant ($p > 0.3$). No consistent long-term changes in oxygen concentrations (three periods of three min before turning DBS on and after an interval of 20 min three periods of three min before novelty exposure) were observed (Fig 5B).
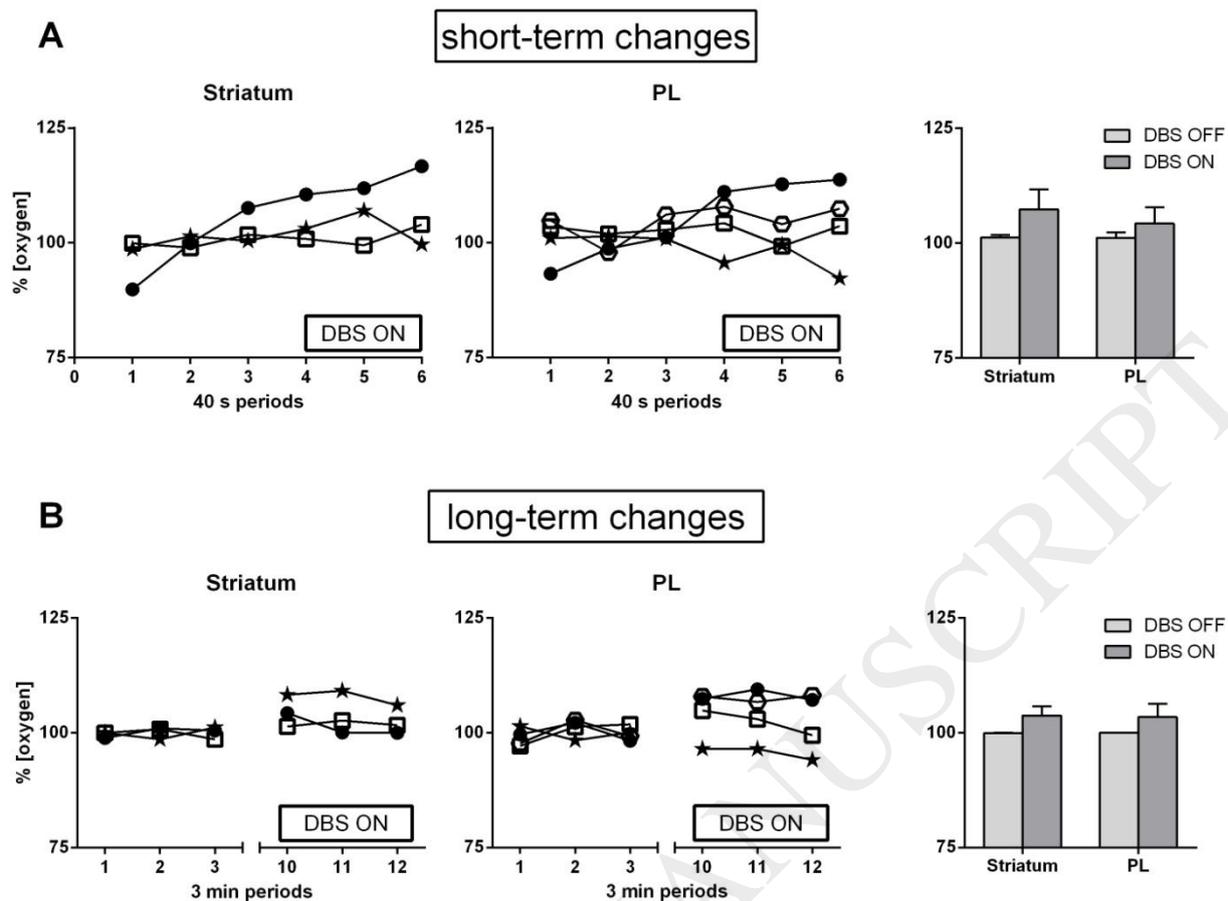
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Figure 5. *DBS effect on basal oxygen levels.*

The effects of turning on DBS in the internal capsule on normalized oxygen concentrations (± SEM) in the prelimbic cortex (PL, n=4)) and the striatum (n=3) are presented as immediate, short-term changes (A) and as long-term changes (B). For short-term changes, oxygen measurements were averaged over six subsequent periods of 40 s and DBS was turned on between the third and the fourth period. For long-term changes, measurements were averaged over 3-min periods, directly before DBS was turned on and 20 min after that, just before the introduction into the novel environment.

3.3 DBS effect on prelimbic cortical and striatal oxygen levels during novelty exposure and restraint.

Behavioral arousal was induced by exposing animals to environmental novelty. Oxygen levels were recorded in PL and striatum on two subsequent days, during sham stimulation or during DBS. After a stable baseline was reached, mice were placed into a new cage containing a piece of wood, a plastic

toy, and bedding. A few seconds after releasing animals into the novel cage, an increase in motor, rearing, and sniffing activities, typical of exploration, was observed.

Two minutes of raw data, consisting of 1200 consecutive current measurements, immediately before the novelty exposure (Figure 6A) or the restraint (Figure 6B), was used as baseline. Changes in oxygen during the tests were evaluated by averaging 1200 consecutive current measurements as a stable current was reached. Fifteen minutes after placement in the novel cage, oxygen levels returned to baseline, and restraint stress test was administered. Animals were placed on a metal grid, movements were reduced by hand for 5 minutes, and then mice were released into their home cage.
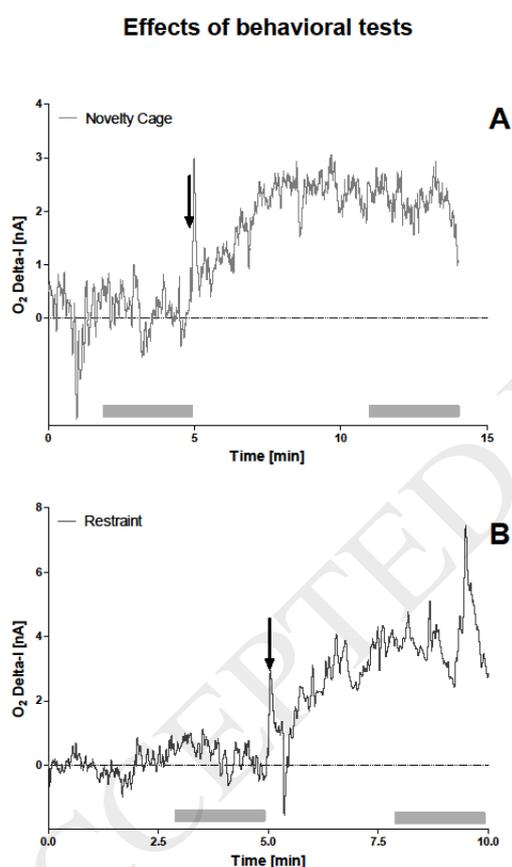

Figure 6. *Effects of behavioral tests on brain oxygen.*

Representative response of oxygen current to novelty exposure and restraint test. Behavioral arousal and stress were induced in mice by exposing animals to environmental novelty (A) and to restraint (B) resulting in increased oxygen currents. Black arrows indicate the beginning of behavioral tests. Gray bars = 2 minutes (1200 consecutive current measurements) used to calculate baseline and behavioral tests effect on brain oxygen. Currents are given as Delta - I, baseline-subtracted current.

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The normalized oxygen concentrations during this experiment are shown in Figure 7. The responses were very consistent: in all individual cases oxygen increases were observed after novelty or restraint compared to the respective baseline periods. This includes the animal in which the baseline was increased on the second day. An overall statistical analysis showed highly significant effects of time for both PL ($F_{2,9}$ = 50.015, p < 0.001) and striatum ($F_{2,6}$ = 23.982, p = 0.009), while a significant effect of DBS was observed in the striatum only ($F_{1,6}$ = 41.525, p = 0.023). Contrast analysis suggested significant increases of both novelty exposure and restraint in both areas (p < 0.05, Sidak-corrected). During sham stimulation novelty and restraint increased PL oxygen levels by 20 and 29%, respectively, while in the striatum the increases were 25 and 22%, respectively (Figure 8). To compare the effects of these two stimuli, we performed a repeated measures analysis of the relative increases, which resulted in a significant main effect of stimuli in the PL ($F_{1,3}$ = 10.526, p = 0.048), indicating that in the prefrontal area restraint more strongly elevated activity than novelty.
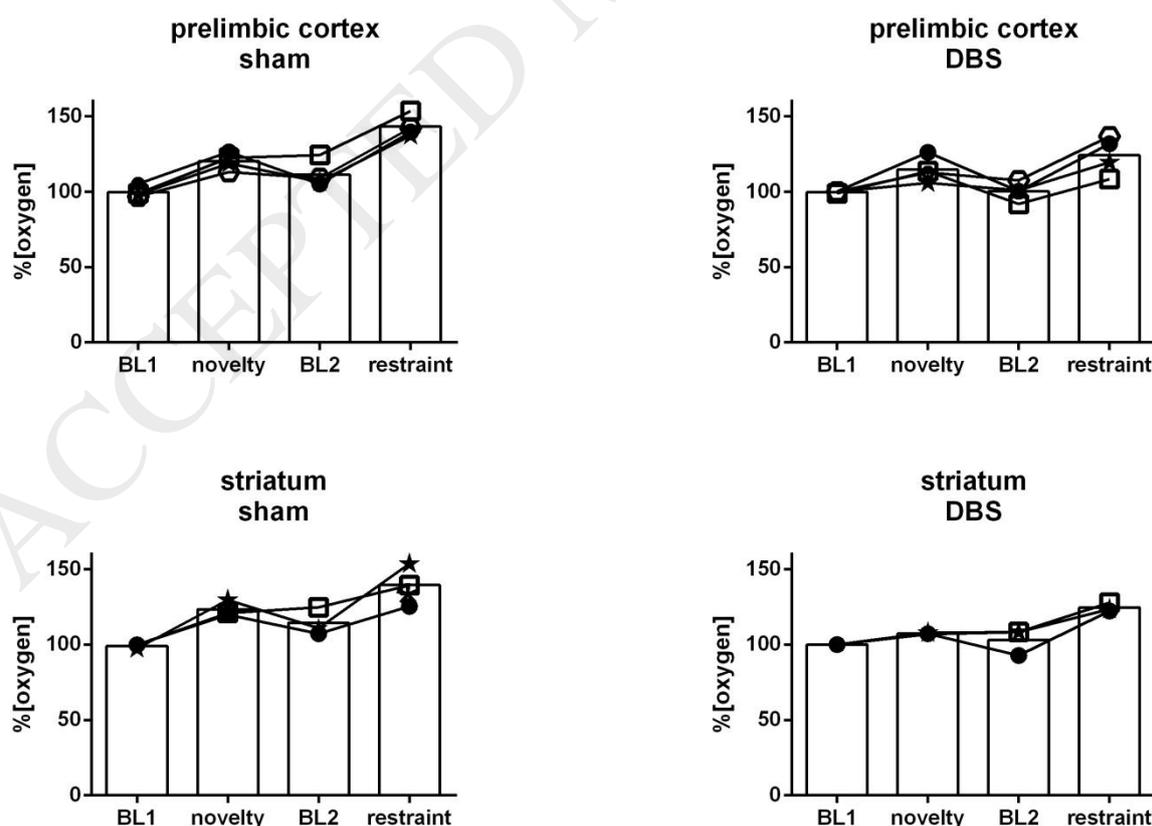
Figure 7. Effect of exposure to stimuli on oxygen concentrations in the prelimbic area of the prefrontal cortex (PL) (n = 4) and the striatum (n = 3) in the absence and presence of DBS in the internal capsule. All values represent the average oxygen concentration (± SEM) in a 2-min baseline period and during exposure to novelty or manual restraint (as indicated by gray bars in Figure 7). Values are expressed relative to the full 10-min baseline period before the novelty exposure.

To analyze the interaction of the stimulus effects and DBS, we compared the percentage of change in oxygen concentrations on the sham-stimulation and DBS days (Fig.8). The most consistent effect was that DBS decreased the novelty-induced increase in the striatum in all cases. Separate analyses of the effects of novelty and restraint showed a significant interaction of novelty and DBS ($F_{1,2}$ = 21.345, p = 0.044) in the striatum. DBS significantly reduced the novelty-induced increase from 24.7 ± 4.2 % to 7.3 ± 0.3% in the striatum ($F_{1,2}$ = 19.185, p = 0.048) (Figure 8).
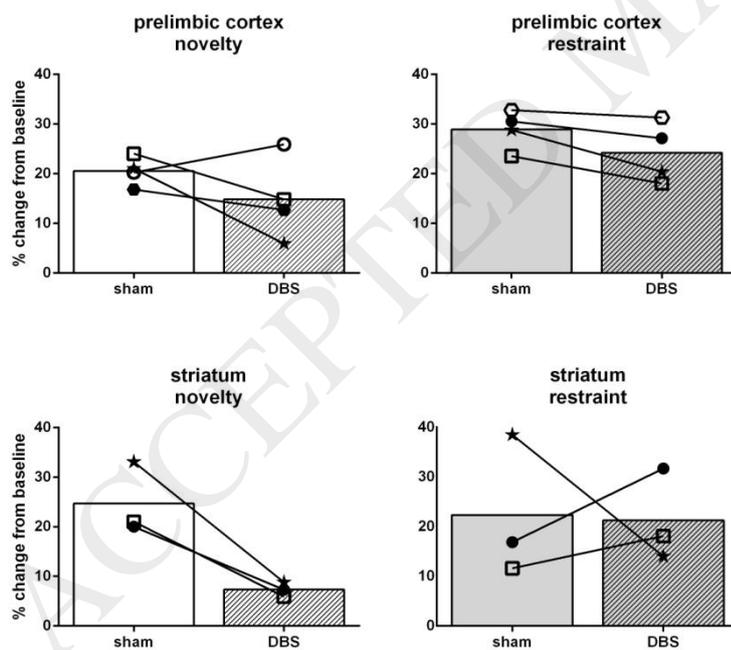

Figure 8.

Novelty- and restraint-induced increases in prelimbic cortical (PL) and striatal oxygen concentrations during DBS and sham stimulation of the internal capsule in freely moving mice.

Shown is the within-animal comparison of increases in normalized oxygen concentrations on the two subsequent experimental days (PL, n = 4; striatum, n = 3). DBS and sham stimulation were applied in a counterbalanced order on these two days.

4. Discussion

We describe the successful combination of bilateral DBS with amperometric oxygen measurements in multiple brain areas of freely moving mice during exposure to behavioral stimuli. Oxygen measurements were stable and DBS did not interfere with oxygen detection. By applying a two-day counterbalanced experimental protocol with DBS on and off, we enabled within-animal control measurements. Exposure to a novel environment and manual restraint resulted in robust and reproducible increases in oxygen levels in both the prelimbic area of the prefrontal cortex (PL) and the striatum. We observed a selective inhibitory effect of DBS in the internal capsule (IC) on the striatal response to novelty. Together, the combination of DBS and oxygen measurements provides a promising technique to study the effects of DBS on brain activity in mice models of psychiatric and neurological disorders.

Carbon-based sensors are routinely used for oxygen detection in the brain of freely moving rodents (Bolger & Lowry, 2005; Serra et al, 2010). These measurements provide an animal surrogate of human fMRI BOLD recordings of cerebral activity (Lowry et al, 1997; 2010; Francois et al, 2012). However, the application of such oxygen measurements has up to now been restricted to behavioral and pharmacological studies, and not to determine the effects of neuromodulatory interventions such as DBS. An urgent question in translational neuroscience is how DBS modulates the activity of brain circuits to achieve therapeutic effects in patients with advanced and/or therapy-refractory neurological of psychiatric diseases (Denys et al, 2012; Lozano & Lipsman, 2013). The availability

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of a method that probes the same measure for brain activity in rodents and humans would allow direct comparisons of DBS-induced effects on brain circuits and provide better insight in the way in which DBS modulates brain circuits. Up to now, this has not yet been described. Therefore, we set out to test if DBS effects could be studied using amperometric measurements of brain oxygen in mice.

The combination of two oxygen sensors and two DBS electrodes was well accommodated by mice, and the animals showed normal, spontaneous behavior during the measurements. Baseline oxygen concentrations were in the range of what was previously reported for implanted microelectrodes in rats (Bolger & Lowry, 2005). The concentrations were stable over a two-hour measurement period and in the majority of animals also across two experimental days.

DBS of the (ventral anterior limb) of the IC is clinically used in the treatment of therapy-refractory psychiatric disorders, including obsessive-compulsive disorder (Alonso et al, 2015), and is reported to reduce anxiety and improve mood within minutes following stimulation onset (Denys et al, 2010; de Koning et al, 2016). The IC is a white matter structure predominantly holding fibers from cortical and striatal origin running to more caudal areas (Tulloch et al, 1978; Coleman et al, 1997; Lehman et al, 2011). We used the IC as a target for DBS in mice, as our previous research showed that DBS of the IC strongly reduced the time spent compulsively grooming in Sapap3 mutant mice (Pinhal et al, 2018). DBS was found to involve antidromic activation of passing fibers or nerve terminals (McCracken & Grace, 2007; Gradinaru et al, 2009) and, therefore, our measurements were in PL and striatum. In line with a possible antidromic stimulatory effect, our previous studies showed increased cFos-expression in PL and other prefrontal areas after IC stimulation in Sapap3 mutant mice (Pinhal et al, 2018).

Although average results suggested a small increase, no significant short-term (up to 9 min; Fig.6a) or long-term (up to 60 min; Fig.6b) effects were observed of turning on the stimulation. This is in apparent contrast to our previous results with cFos and also to a number of previously reported increases in BOLD or CBV responses in brain areas distant from various DBS (Albough et al, 2016; Chao et al, 2014; Lai et al, 2013; Van Den Berge et al, 2017; Young et al, 2011). However, we must

take into account that the IC was not among the previously studied DBS targets, that it is not yet known if there is a difference in sensitivtiy between responses of fos-expression and of oxygen concentrations and that the fos-response was obtained in an animal model of compulsive behavior – it may well be that DBS specifically acts on pathological network activity in affected neuronal circuits (see Introduction). On the other hand, the use of awake, freely moving animals may be expected to lead to even stronger fMRI responses because DBS effects in awake, immobilized rodents were reported to be stronger than in sedated or anesthetized animals used in most studies (Tsurugizawa et al, 2010; Desai et al, 2011; Lahti et al, 1999). Therefore, taken all this into account and given the variability of the individual responses (see Fig.5), it seems possible that the main reason we cannot report significant increases is that baseline oxygen measurements show more fluctuations and more interindividual differences in freely moving compared to immobilized and sedated mice and that the use of a larger group size is indicated in follow-up experiments.

To study the neuromodulatory effects of DBS on dynamic changes in regional oxygen concentrations, we used two behavioral stimuli. Exposure to novelty is a widely used, physiological stimulus that elicits arousal and exploration in animals (Hennessy & Levine, 1979; Kelley et al, 1989). We observed similar increases in oxygen concentrations in PL and striatum in mice placed in a novel environment. This is in accordance with the effects of novelty exposure on neuronal activity as visualized by cFos expression (Handa et al, 1993), although some researchers reported that relatively more salient novel experiences were required for striatal cFos activation (Muigg et al, 2009; Rinaldi et al, 2010). The other stimulus, manual restraint, also induced increases in oxygen in both PL and striatum, in accordance with its effects on cFos expression (Cullinan et al, 1995; Kim et al, 2014). However, restraint is a more stressful experience than novelty exposure, e.g. resulting in higher increases in plasma corticosterone (Lau, 1992). This was reflected in the restraint-induced increases in oxygen in PL which were significantly higher compared to novelty exposure (increases of 29% vs 20%, respectively). Interestingly, the striatal responses were not different (increases of 22% vs 25%). A similar contrast was also reported for *in vivo* dopamine release in rats and mice (Feenstra et al,

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2000; Ihalainen et al, 1999), suggesting that it might be interesting to study a possible relation between these responses.

A remarkable result is the selective DBS effect on the novelty-induced oxygen response in the striatum, but not PL. This cannot be due to a habituation (or sensitization) to novelty as we used a counterbalanced set-up of the experimental sequence of sham stimulation and DBS. The difference between the change in striatal effects to novelty and the relative stability of the other responses speaks against this, as well.

We can only speculate as to why DBS in the IC selectively counteracted the novelty reponse in the striatum. We referred to the indications that for a striatal response a more salient novelty experience is needed (Muigg et al, 2009; Rinaldi et al, 2010). DBS may have reinforced this difference and it should be interesting to study the functional connectivity between the prefrontal and striatal areas – the selective change in striatal activity suggests that the connectivity may be altered during DBS. As effects on functional connectivity between cortical and striatal areas have been shown to be associated to clinical improvements in obsessive compulsive disorder (OCD) (Figee et al, 2013), the presently proposed methodology might be used to study the mechanism of action of DBS in animal models for OCD, such as the Sapap3 mutant mouse (Welch et al, 2007).

The present study has a number of potential limitations. First, the number of mice was low. Still, the results of the stimulus-induced changes and the effects of DBS on these changes were so consistent that we feel confident to conclude that the combination we tested presents a valuable contribution to the field. Second, the stimuli we tested were simple and not specifically designed to test pathological emotional or cognitive processes, while we also used normal control animals to test DBS effects. Yet, a similar set-up would also be suitable to determine DBS effects on e.g. the compulsive behavior of the Sapap3 mutant mice we used before (Pinhal et al, 2018). Moreover, others have shown that sensor-based brain oxygen measurements during Pavlovian conditioning are possible in mice (McHugh et al, 2014).

5. Conclusion

Amperometric measurements of brain oxygen to assess effects of DBS provides several advantages compared over the use of rodent fMRI. Here, we show that it is possible to monitor DBS effects on oxygen responses in awake, freely moving animals across several days. Moreover, in our experience the implementation of this method was relatively easy and rapid (several weeks), while the combination with DBS proved considerably less complicated than in the case of fMRI. The relatively low equipment cost and the straightforward data analysis are additional points in favor of the use of oxygen sensors. The method offers opportunities for comprehensive and chronic studies of DBS effects on brain activity in freely-moving rodents. This may be of particular importance in mouse models for movement disorders such as Parkinson's disease and psychiatric diseases characterized by compulsive activities such as OCD. Simultaneous recordings of brain activity and unrestricted movements will provide novel information of translational importance, making this a potentially very powerful method.

Conflicts of interest

None of the authors have any conflicts of interest associated with this study.

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