Animal studies in clinical MRI scanners: A custom setup for combined fMRI and deep-brain stimulation in awake rats

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A B S T R A C T
Background: In humans, functional magnetic resonance imaging (fMRI) cannot be used to its full potential to study the effects of deep-brain stimulation (DBS) on the brain due to safety reasons. Application of DBS in small animals is an alternative, but was hampered by technical limitations thus far.

New Method: We present a novel setup that extends the range of available applications by studying animals in a clinical scanner. We used a 3 T-MRI scanner with a custom-designed receiver coil and a restrainer to measure brain activity in awake rats. DBS electrodes made of silver were used to minimize electromagnetic artifacts.

Results: Using our novel setup, we observed minor DBS-electrode artifacts, which did not interfere with brain-activity measurements significantly. Movement artifacts were also minimal and were not further reduced by restrainer habituation. Bilateral DBS in the dorsal part of the ventral striatum (dVS) resulted in detectable increases in brain activity around the electrodes tips.

Comparison with Existing Methods: This novel setup offers a low-cost alternative to dedicated small-animal scanners. Moreover, it can be implemented in widely available clinical 3 T scanners. Although spatial and temporal resolution was lower than what is achieved in anesthetized rats in high-field small-animal scanners, we obtained scans in awake animals, thus, testing the effects of bilateral DBS of the dVS in a more physiological state.

Conclusions: With this new technical setup, the neurobiological mechanism of action of DBS can be explored in awake, restrained rats in a clinical 3 T-MRI scanner.

1. Introduction

Deep-brain stimulation (DBS) is an intervention that relies on implanted electrodes delivering high-frequency currents into targeted brain areas. It is increasingly being examined as a treatment for neuropsychiatric disorders (Harmsen et al., 2020), such as obsessive compulsive disorder (Denys et al., 2020), major depressive disorder (van der Wal et al., 2020), and drug and alcohol addiction (Bari et al., 2018). While there is still a limited understanding of the underlying mechanisms of action of DBS and its therapeutic effect, it is often hypothesized that DBS results in restoring normal activity and functional connections in specific neural circuits (Bourne et al., 2012; Figee et al., 2012).

With functional magnetic resonance imaging (fMRI) it is possible to investigate DBS-induced regional as well as global changes in brain function. However, fMRI in patients with DBS implants is a safety hazard. The interaction between the MRI scanner’s radio frequency (RF) fields and the implanted electrodes, the conductive leads, and other DBS devices, can induce electric currents on the lead wires, which increase the energy absorption in the tissue surrounding the DBS electrodes (Golestanirad et al., 2019). Furthermore, the static magnetic field and gradient system of the MR machine might cause movement of the implanted electrodes or malfunction of the DBS device. Even though...
improvements on this matter have been made, for instance, by reducing the amount of ferromagnetic material used and the programming of specific absorption rate (SAR) reducing MR-sequences, MRI in DBS patients at 1.5 T and pulse-sequences with limited RF power are recommended (Medtronic, 2015).

Where MRI studies in DBS patients suffer from electrode imaging artifacts because of the materials used, and in addition are limited to low-field MRI scanners with the DBS turned off during acquisition due to safety considerations (Tagliati et al., 2009; Medtronic, 2015), studying the effects of DBS on the brain in a rodent model has several advantages. For instance, combining DBS and fMRI in an animal model allows targeting of different and multiple brain areas, varying the properties of the DBS current applied to the brain, and comparing effects in disease models and normal controls (Feenstra and Denys, 2012). In addition, higher filed strengths can be applied.

In the present study, we describe a novel DBS-MRI setup for investigating the effects of DBS on brain activity in awake rats, using fMRI. Our overall aim is to use fMRI in awake animals to study the effects of high-frequency DBS in different target locations, such as the ventral striatum/nucleus accumbens and the internal capsule (Denys et al., 2020; van Dijk et al., 2013; Pinhal et al., 2018). Here, we present our findings using the novel setup while using the dorsal part of the ventral striatum (dVS) as target for DBS.

Scanning awake animals is an advantage because anesthetics have been reported to suppress and alter neural activity and functional connectivity (compared to awake animals), while increasing cerebral blood flow because of the vasodilatory effect of the anesthetics (Pan et al., 2015; Paasonen et al., 2018). Different anesthetics tend to uniquely modulate functional connectivity in MRI settings, all distinct from that in awake animals (Paasonen et al., 2018) possibly reducing the translational nature of these experiments (Chuang and Nasrallah, 2017). For these reasons, and with the reported possibility of immobilizing rodents without overt signs of stress (King et al., 2005; Febo, 2011; Yee et al., 2016), we decided to perform fMRI acquisition in awake rats that were restrained in a custom-designed combination of a body tube, head-stage and nose cone. We have adopted a training protocol aiming to habituate the animals to, and minimize stress caused by the restraining and MRI environment. The effectiveness of our restraining training protocol was examined by looking at the changes in blood-plasma corticosterone levels over time and by comparing motion parameters during fMRI acquisition between trained and untrained animals.

We explored the possibilities of using a standard 3 T clinical MRI scanner. The choice for a clinical MRI scanner was prompted by our wish to perform fMRI experiments in rats when access to a high-field small animal scanner is not available. This meant that we had to use a newly designed MRI receiver coil intended for scanning rat brains. This two-channel surface coil consists of three coil loops covering the top and sides of the rat brain and enables parallel imaging. The coil fits closely around the head of the animal within the restrainer tube. Both the restrainer and coil designs incorporate special openings for connecting gas tubes and wires.

As our standard platinum/iridium DBS electrodes are suboptimal for use in an MRI scanner, we used custom-made, MRI compatible electrodes made of 99.99 % pure silver wires. The MRI artifacts generated by magnetic susceptibility of our silver electrodes were compared to the artifacts seen when using platinum/iridium wires, a material commonly used in preclinical animal research and some patient DBS systems (Medtronic, 2009). We assessed the effectiveness of our custom-made electrodes by comparing cFOS expression at the electrode tip of an active DBS electrode in the dVS, with that of a non-active electrode.

With this setup, we have successfully acquired BOLD fMRI measurements of bilateral DBS, in animals that were awake during data acquisition, and with minimal electrode artifacts.

2. Methods and analyses

2.1. Electrodes and fabrication

Stimulation electrodes were custom-made from either platinum/iridium wires (90 % platinum, 10 % iridium, 75 μm bare diameter, 18 μm PFTE insulation coating, Advent Research Materials LTD, Oxford, United Kingdom), a paramagnetic alloy commonly used for electrodes in animal research with a magnetic susceptibility of $27 \times 10^{-5}$ at 20 degrees Celsius, or silver wires (99.99 % pure silver, 125 μm bare diameter, 26 μm PFTE insulation coating, Advent Research Materials LTD, Oxford, United Kingdom), a diamagnetic alloy with a relatively lower magnetic susceptibility of $-2.6 \times 10^{-5}$.

The wires were twisted into a two-channel electrode lead and cut to the appropriate length, with a distance of approximately 250 μm between the two contact points in the longitudinal direction in order to enlarge the stimulation area and prevent short-circuiting (Fig. 1A&C). From each contact point, approximately 200 μm of insulation material was removed to enlarge the contact surface. At the opposite end, the electrode connector was created by stripping approximately 3.5 mm of the leads’ insulation and inserting each end into a 7 mm long sterling silver tube (92.5 % silver, 7.5 % copper, 1.5/1.0 mm outer/inner diameter, Bijou Moderne, Bleiswijk, the Netherlands). The end of the tube where the lead was inserted into was closed off with pliers; the flattened tube end now clasping onto the lead inside and ensuring electrical conduction, and the open tube end serving as a connector. Both silver tubes were then glued into a small plastic block holding them in place (Fig. 1).

The impedance of each electrode was tested by placing the electrode tips in saline solution (0.9 % sodium chloride in water) while connected...
to an impedance meter (IMP-1, BAK Electronics, Inc., Umatilla, Florida, United States). Measured impedances varied between 10 and 20 kΩ at 1 kHz.

2.2. MRI

In order to determine the MRI artifacts due to the magnetic susceptibility of the different materials, the electrodes were lowered into a small container completely filled with saline solution (0.9% sodium chloride in water) which was closed off. T2-weighted images were obtained using a multi-shot turbo spin echo (TSE) sequence (TSE factor = 8, TR = 4000 ms, TE = 56 ms, acquired voxel size = 0.2 × 0.2 × 1.5 mm, acquired matrix size = 176 × 168, 12 slices). The images were acquired on a 3.0 T Philips Ingenia MR system (Philips Healthcare, Best, the Netherlands) using the custom-made two-channel receiver coil (MRCoils, Zaltbommel, the Netherlands), part of the restrainer setup described below.

2.3. cFOS

2.3.1. Subjects

Fifteen male Wistar rats (225 – 250 grams, Charles River Laboratories, Saint-Germain-sur-l’Arbresle, France) were included in this study. Four animals were excluded on basis of incorrect electrode placement. The animals were housed socially during the acclimatization period of one-week minimum before surgery and individually after surgery, on a normal day-night cycle (lights on 7am to 7pm) with ad libitum food and water. The experiments were approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences (KNAW) and carried out in agreement with Dutch law (Wet op de Dierproeven, 1996) and European regulations (Guideline 2010/63/EU).

2.3.2. Surgery

For electrode implantation, animals were put under isoflurane anesthetics (3% induction, 1.8-2.5% maintenance, 1:1 air in oxygen at 0.3 L/min, Isoflo, Zoetis, Rotterdam, the Netherlands) and their temperature was kept at 37 degrees Celsius by means of a heating pad. After a sagittal incision, the skull was exposed and two Burr holes were drilled to allow for bilateral electrode implants. Two custom-made silver electrodes were lowered into the brain, targeting the dVS (AP + 1.72 mm, ML +/-1.60 mm from bregma and DV – 7.0 mm) (Paxinos and Watson, 2007). Four stainless steel screws were placed rostrally and caudally to the electrodes, to secure the electrodes to the skull with acrylic dental cement (Simplex Rapid, Kemdent, Swindon, United Kingdom). Then, the wound was closed with sutures and the animals were given a subcutaneous meloxicam injection (1 mg/kg, Meloxicam, Boehringer Ingelheim Vetmedica Ltd., Duluth, Georgia, United States) for post-op pain management. The animals were allowed a minimum recovery time of one week.

2.3.3. Unilateral DBS

To protect the leads connected to the DBS electrodes from being damaged by the animals or getting intertwined, animals were tethered to a counterbalanced arm, part of a Stand-Alone Raturn System (BASi, West Lafayette, Indiana, United States), allowing the animal to move freely inside a plastic bowl with cage bedding and access to water, in a non-illuminated box. Both implanted electrodes were connected to a digital stimulator (DS8000, World Precision Instruments, Berlin, Germany) through constant current digital linear stimulation isolators (DSL100, World Precision Instruments, Berlin, Germany) with carbon fiber wires (CPVC4050, 18 American wire gauge, 1.0 mm diameter, 3000 strands, World Precision Instruments, Berlin, Germany). If the impedance of the left hemisphere electrode did not exceed 50kΩ, it received biphasic, constant current pulses (80 μs pulse width at 130 Hz, 300 µA stimulation, as described in van Dijk et al., 2011) for 30 minutes continuously, with the right electrode functioning as sham. If the left hemisphere electrode did exceed 50kΩ, sham and stimulation electrode sides were reversed. Ninety minutes after DBS, the animals were deeply anesthetized with sodium pentobarbital (100 mg/kg) and transcendally perfused with phosphate-buffered saline (PBS) and fixated with 4% paraformaldehyde (PFA). The brain was put in PFA for 24 hours, transferred to a 30% sucrose in PBS solution until saturated, and flash frozen in isopentane (HoneyWell, Seelze, Germany) before stored at -80 degrees Celsius.

2.3.4. cFOS immunohistochemistry

Coronal sections of the animals’ brains were taken using a cryostat (Leica Biosystems, Wetzlar, Germany), collected free-floating in cryoprotectant (30% glycerol, 30% ethylene glycol, 40% 0.1 M PBS), and stored at -20 degrees Celsius. Sections containing the electrode tips were selected for cFOS immunohistochemistry, with two consecutive slices per electrode tip. The sections were stained using the avidin-biotin complex (ABC) method. First, the sections were pretreated for ten minutes in a solution of 10% methanol (HoneyWell, Seelze, Germany) and 3% H2O2 (30%, Merck, Darmstadt, Germany) in TBS to suppress endogenous peroxidase. Then, after immersing them in blocking solution (10% horse serum (Invitrogen, Carlsbad, California, United States) in SuperMix (SUMI, 0.5% Triton-X 100 (Sigma-Aldrich, Zwijndrecht, the Netherlands) and 0.25% gelatin in TBS)), the sections were incubated in a solution of the primary antibody, rabbit anti-cFOS (1:5000, ab190289, Abcam Biotechnologies, Cambridge, United Kingdom), and 4% horse serum in SUMI for 24 hours at 4 degrees Celsius; then incubated in a solution of the secondary antibody, biotinylated horse-anti-rabbit IgG (1:600, Vector Laboratories Inc., Burlingame, California, United States), and 4% horse serum in SUMI for 1 hour; then placed in a avidin-biotin horseradish peroxidase complex solution (1:800, ABC Elite Kit, Vector Laboratories Inc., Burlingame, California, United States) for one hour for signal amplification; and finally incubated in a solution of diaminobenzidine (DAB, 0.05%, Sigma-Aldrich, Zwijndrecht, the Netherlands) with 0.03% H2O2 and 0.23% nickel/ammonium sulphate.
implants: 1- Silver connectors of two bilaterally implanted custom-made silver electrodes, 2- Plastic connector for head fixation. C) Close-up front view: 1- shoulder coil (see Fig. 4 for more details), 5- shoulder bar to restrict forward movement (black), 6- clear body tube, 7- disc to restrict backward movement (white). B) Head adjustable horizontal bar (black) for head fixation, 3- fully adjustable nose piece including bite bar and nose cone for isoflurane supply (blue), 4- custom made MRI mountant (Merck, Darmstadt, Germany).

2.3.5. cFOS detection

The sections were digitized at 10x magnification with a CCD camera (D-sight bright-field slide scanning microscope, A. Menarini Diagnostics, Italy) at equal lighting settings between images. Analyses were performed using Image Pro (Image Pro Plus, Media Cybernetics Inc., Rockville, Maryland, United States). A circular ROI of a fixed size (350 μm diameter) was manually placed around the electrode tip, and a background measurement area was set over white matter. A low-pass filtered image was created from the green channel of the 8-bit RGB image, in which pixels that exceeded the optical density (OD) threshold (set to produce the optimal SNR in relation to the background area) were marked as cFOS positive. Correction for difference in background staining between sections was done by calculating the relative density (RD): if the OD measured in the background would exceed the OD threshold, the background OD was subtracted from the OD in the ROI. The RD was averaged over the two selected section per animal, for each ROI.

2.3.6. Analysis

The difference between the cFOS expression in DBS versus sham stimulated hemispheres was analyzed by performing a paired samples t-test on the average RDs, obtained as described above (n = 9). The analysis was done using the open-source statistics software JASP (JASP Team, 2019, version 0.9.1).

2.4. Restrainer setup

The custom-build, MRI-compatible restrainer setup, was designed for the purpose of MR image acquisition of the brains of awake rats, while retaining access to the animal’s head and tail (Fig. 3). This offers a wide variety of experimental possibilities during MRI acquisition, such as electrical or optical stimulation, access to cranial windows and/or infusions into or sampling from the brain, as well as from the tail.

The restrainer setup consists of three main parts – a body tube, a bite bar with anesthetics nose cone, and a fully adaptable head stage holder – which are placed onto a clear plastic base plate. Inside the setup, a custom-made two-channel surface receiver coil array (MRCoils, Zaltbommel, the Netherlands) can be placed and secured (Fig. 4). The restrainer setup consists of two loops, one covering the top of the rodent’s head (channel one). The second loop (channel two) is created by connecting two loops located to the left and right side of the rodent’s head with copper wiring, effectively forming a single loop. The connecting copper wiring of the second loop creates a 10-percent overlap with the first, decoupling the two channels. Together, the coil loops’ coverage encompasses the entire brain of the rodent. The coil loops were placed on the inside of an acrylic arch, with an opening in the top, that will fit tightly around an adult rat’s head. Coil tuning is achieved by fixed capacitors, while matching can be done with variable capacitors on-site if needed. The leads running from the surface coil are covered by ceramic floating cable traps, suppressing shield currents. The coil can be connected to the scanner-bed interface through a custom-made interface box (MRCoils, Zaltbommel, the Netherlands). This sixteen-channel interface box, containing low noise factor amplifiers and transmit/receive switches, serves to connect custom made MRI coils through BNC-connectors. The interface box itself connects to the scanner bed

Fig. 3. Custom-made MRI restrainer setup for simultaneous scanning and DBS in awake rodents. A) Top-down view: 1- oxygen and isoflurane supply-hose, 2- adjustable horizontal bar (black) for head fixation, 3- fully adjustable nose piece including bite bar and nose cone for isoflurane supply (blue), 4- custom made MRI coil (see Fig. 4 for more details), 5- shoulder bar to restrict forward movement (black), 6- clear body tube, 7- disc to restrict backward movement (white). B) Head implants: 1- Silver connectors of two bilaterally implanted custom-made silver electrodes, 2- Plastic connector for head fixation. C) Close-up front view: 1- shoulder bar to restrict forward movement, 2- clear body tube, 3- screw running through the adjustable horizontal bar, used to attach and fixate animal’s head, 4- electrode connectors, 5- custom-made MRI coil, 6- animal’s nose protruding into the nose cone (blue).

Fig. 4. Custom-made two-channel head surface coil for rats. A) The coil inside its casing. Electrode and head-fixation connectors protrude through an access hole (arrow), allowing the coil to be lowered fully onto the animal’s head. B) Top-down view of the coil, with casing removed: 1- access hole, 2- the coil loop covering the top of the rodent’s head. C) Inside, bottom-up view of the two-channel surface coil. Arrows indicate the placement of the three coil loops constituting the coil array: 1- loop covering the top of the rodent’s head (channel 1), 2 and 3- loops covering the sides of the rodent’s head (channel 2). D) Side view: 1- the coil loop covering the top of the head, 2- the coil loop covering the left side of the head (same as right side).
interface. The MR scanner’s body coil was used as the transmit coil.

The animal’s body is secured into place inside a clear plastic tube. After the animal’s front and hind paws are fixated against its body with adhesive tape or a Rodent Snuggle (Lomir Biomedical Inc, Notre-Dame-de-l’Ile-Perrot, Canada), it can be put into the body tube where a plastic disc closes off the tube at the rear of the animal, preventing the animal to move backwards. The animal’s tail is put through the hole in the disc, and the disc is placed against the animal’s rear and locked in place with a screw. The hole in the disc leaves room for tubing or wires next to the tail. At the other end of the body tube, the animal’s head is sticking out from the neck up. A u-shaped plastic rod is put through the tube, from top to bottom, in between the head and shoulders of the animal on the left and right side, preventing the animal from moving forwards or backwards. The tube is then placed onto the base plate: holes in the bottom of the tube align with screws on the base plate and the tube can be screwed down.

The animal’s incisor teeth are placed over a bite bar. This piece of 3D-printed plastic is designed after a typical stereotaxic bite bar, and can be moved forwards, backwards and up and down. A 3D-printed nose cone can be placed over the animal’s nose, to provide inhalation anesthetics or other gasses. After fixating the animal’s incisors properly inside the bite bar it can be locked into place with screws. The part of the bite bar that actually gets covered by the incisors, can easily be taken out and replaced if damaged.

The custom-made, two-channel arch-shaped receiver coil is then lowered onto the animal’s head and secured into the setup with screws on the left and right side. The coil can be moved down as to touch the head of the animal; the body tube and the bite bar can be moved forwards or backwards to locate the animal’s head exactly in the center of the coil. In the top of the coil is a 1.5x1.0 cm large opening, allowing access to the animal’s skull. There is room for items protruding from, or being lowered into the skull, such as cannulas or connectors.

Finally, the animal’s head is secured into place. This is done by attaching a head stage (a 3D-printed plastic block which is attached onto the animal’s skull) to the head stage holder of the restrainer setup. The head stage holder is composed of four vertical carbon fiber rods, two on the left and two on the right of the setup, connected by two horizontal, plastic, 3D-printed beams. From the center of those beams, a single carbon fiber rod runs from left to right with the center of the rod located over the animal’s head. This rod can be lowered onto the animal’s head, and a plastic hood, glued into the rod, can be placed over the head stage that is fixed onto the animal’s skull and locked down with a plastic screw. The head stage holder is fully adjustable and can be moved in all directions to fixate the animal’s head and align it correctly for MR image acquisition.

After the animal is fixated in the restrainer setup, the whole setup can be placed on the scanner bed and put inside the bore. By placing sandbags against the sloping sides of the base plate, movement of the setup inside the scanner can be prevented.

2.5. Restraining habituation and corticosterone

2.5.1. Subjects

Fourteen male Wistar rats (250 – 350 grams, Charles River Laboratories, Saint-Germain-sur-l’Arbresle, France) were included in this study. The animals were housed socially during the acclimatization period of one-week minimum before surgery and individually after surgery, on a normal day-night cycle (lights on 7am to 7pm) with ad libitum food and water. The experiments were approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences (KNAW) and carried out in agreement with Dutch law (Wet op de Dierproeven, 1996) and European regulations (Guideline 2010/63/EU).

2.5.2. Surgery

For placing and securing a plastic, MRI-compatible pedestal to the animals’ skull, animals were put under isoflurane anesthetics (3% induction, 1.8-2.5% maintenance, 1:1 air in oxygen at 0.3 L/min, Isoflo, Zoetis, Rotterdam, the Netherlands) and their temperature was kept at 37 degrees Celsius by means of a heating pad. After a sagittal incision, the skull was exposed, the plastic pedestal was placed onto the skull and two burr holes were drilled to allow for plastic, MRI-compatible screws to lock the pedestal in place using the screw holes of the pedestal. A third screw was placed caudally to the pedestal, and acrylic dental cement (Simplex Rapid, Kemedent, Swindon, United Kingdom) was used to secure the pedestal in place. Then, the wound was closed with sutures and the animals were given a subcutaneous meloxicam injection (1 mg/kg, Metacam, Boehringer Ingelheim Vetmedica Ltd., Duluth, Georgia, United States) for post-op pain management. The animals were allowed a minimum recovery time of one week.

2.5.3. Restraining-habituation protocol

The training protocol for the purpose of habituating the animals to being restrained, consisted of six consecutive days of the animals being restrained and exposed to MRI noises, aiming at acclimatization to the restraining and noises and thereby reducing the stress the animals experienced during the training. Blood samples were taken at regular intervals, in order to later assess the stress levels of the animals by analyzing the amount of corticosterone in the blood plasma.

Rats were mildly sedated using isoflurane anesthesia to be placed in the MRI-compatible restrainer device (3% induction, 1.5-2.0 % during placement, in medical air (70 % N2O, 30 % O2) at 0.6 L/min). The paws of the animals were fixated against its body before it being placed inside a clear plastic tube. Then the rat was fixated as described before.

After the animal was placed inside the restrainer, a catheter, with a length of clear tubing which could be closed off connected to it, was placed inside the tail vein in order to take blood samples during the training sessions (Vasofix Safety, 22 G, B. Braun Melsungen AG, Melsungen, Germany). At this point the first blood sample was collected (200 μL, indicated as ‘before restraining sample’ as the animal is still anesthetized) in a blood collection capillary tube (Microvette CB 300 Z, Sarstedt AG & Co. KG, Nümbrecht, Germany), and the tubing and dead space of the catheter were flushed with a heparin solution (2.5 I.U./ml in 0.9 % saline solution). Then the animal was placed inside a sound insulated box, in which two audio speakers were placed. Isoflurane anesthetics were turned off, and a second blood sample was taken at this time. By means of an USB camera placed inside the box, the animals could be observed. Ten minutes after the anesthetics were turned off, the animals were awake - as indicated by whisker movements. At this point the third blood sample was taken (indicated as ‘beginning of restraining sample’).

With the animal awake, MRI sounds consisting of the noises of a variety of sequences were played through the speakers inside the box for 60 min. The power level measured at the location of the animal’s head reached a maximum of 100 decibels. During this period, blood samples were collected every ten minutes until the end of the training session (indicated as ‘end of restraining sample’). As the clear tubing connected to the tail vein catheter was running from inside the box to the outside, the box could remain closed throughout the training sessions, and the tubing and dead space of the catheter were flushed after every sampling with a pre-calculated volume of heparin solution to ensure no blood remained in the tubing and catheter. Blood plasma was prepared by centrifuging the blood samples for 15 min at 2000 x g at 4 degrees Celsius. The plasma was stored at -20 degrees Celsius until corticosterone immunoassay.

2.5.4. Corticosterone immunoassay

Plasma corticosterone concentrations were assessed in triplicate using enzyme-linked immunosorbent assay (ELISA) kits (DetectX, K014-H, Arbor Assays, Ann Arbor, Michigan, United States) with a standard range from 10,000 to 78.125 pg/mL assessed in duplicate. A Varioskan microplate reader (ThermoFisher Scientific, Waltham, Massachusetts, United States) was used to acquire the raw optical density (OD) values.
The values for each point in the standard range were averaged, as well as the values for each plasma sample resulting in mean raw ODs. The mean of the blank replicates was subtracted from these mean raw OD values as background correction, resulting in net ODs. Then, with use of a four-parameter logistic curve fit, the plasma corticosterone concentration of each plasma sample was quantified from the net ODs in ng/mL.

2.5.5. Analysis
The effect of the restrainer training was analyzed by looking at the difference in plasma corticosterone concentrations on the first day of training (day 1) and the final day of training (day 6) by comparing the concentrations before restraining (with the animal anesthetized), at the beginning of the restraining and at the end of the restraining session between day 1 and 6, using paired samples t-tests in JASP (JASP Team, 2019, version 0.9.1). Also, the difference in corticosterone concentration from beginning to end of the restraining session was compared between day 1 and 6. This was done by subtracting the concentration measured at the beginning of the restraining session from the end concentration of that session, after correcting for the concentration measured before restraining (\( \frac{\text{end} - \text{beginning}}{\text{end} - \text{before}} \times 100 \)). Then, a paired samples t-test was used to compare the resulting values of day 1 with day 6.

2.6. DBS/MRI experiment

2.6.1. Subjects
Twenty-three male Wistar rats (250–350 grams, Charles River Laboratories, Saint-Germain-sur-l’Arbresle, France) were included in this study. Seven animals were excluded based on incorrect electrode placements, leaving 16 animals. Eight of these animals received training with the purpose of habituating them to MR noises and being restrained (‘trained animals’), while the other 8 did not (‘untrained animals’). The MRI-data of the untrained animals was further analyzed; of those 8 animals, 2 showed non-optimal EPI data and were discarded, leaving 6 animals for the analysis of DBS-induced BOLD activation. The animals were housed socially during the acclimatization period of one-week minimum before surgery and individually after surgery, on a normal day-night cycle (lights on 7am to 7pm) with ad libitum food and water. The experiments were approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences (KNAW) and carried out in agreement with Dutch law (Wet op de Dierproeven, 1996) and European regulations (Guideline 2010/63/EU).

2.6.2. Surgery
The surgery consisted of bilateral electrode implantation into the dVS, and the placement of a MRI-compatible head pedestal on the animal’s skull. The procedures for electrode implantation and placing the pedestal are described in the ‘cFOS’- and ‘Training and corticosterone’-sections respectively – with the exception of the use of stainless steel screws. Here, only plastic screws were used. The animals were allowed a minimum recovery time of one week before taking part in the DBS/MRI experiment.

2.6.3. Restrainer habituation
Leading up to the MRI acquisition, 8 of the animals received restrainer training for 6 consecutive days, as described above. On the day following the last restrainer training, the animals were taken from the stables in the morning and brought to the MRI facility where they remained in their home cages until they went into MRI preparation. The other 8 animals which did not receive restrainer training, were taken to the MRI facility after a minimum one week of recovery from surgery. The batch of 8 trained animals were put in the experiment first, after which the batch of 8 untrained animals followed. All other procedures regarding the DBS/MRI experiment did not differ between the trained and untrained animals.

2.6.4. MRI preparation
The animals were shortly put under isoflurane anesthesia (3%, 1:1 air in oxygen at 0.3 L/min) and placed in the custom-made MRI rat restrainer (see 2.4 Restrainer setup) with the addition of a pneumatic pillow placed under the animal’s body for respiration monitoring (Small Animal Instruments, Inc., Stony Brook, New York, United States). The custom-made, two-channel receiver coil (MRCoils, Zaltbommel, the Netherlands) was placed over the animal’s head, after which the setup was placed inside a 3.0 T clinical scanner (Philips Healthcare, Best, the Netherlands) (Fig. 5). The DBS electrodes were connected to a digital stimulator (DS8000, World Precision Instruments, Berlin, Germany) through constant current digital linear stimulus isolators (DSL100, WPI, Berlin, Germany), both placed outside of the scanner room, connected to the patch bay. Ferrite chokes were used on the cables coming from the stimulus isolators to the patch bay outside the scanner room, to suppress possible high frequency interference. Carbon fiber wires leading from the electrodes to the patch bay were used inside the scanner room.

2.6.5. Practical considerations
When performing MRI scans in awake animals, it is of importance to keep the preparatory period preceding the scans, during which the animals are anesthetized, as short as possible. The animals were secured in the setup on a table with a dedicated isoflurane setup, close to the MRI-room. Preparing the animals for MRI scans was done by two well-trained researchers: one securing the animal inside the setup with the other one assisting. To ensure that fixing the head pedestal to the setup, as well as connecting the leads to the DBS-electrodes was a smooth and easy procedure, the head pedestal was placed as perpendicular to the skull as possible, and a mold the size of the hole in the MRI-coil was used to make sure the placement of the pedestal and electrode connectors stayed within bounds. This way, securing the animal in the setup was done within minutes. Transporting the animal to the MRI scanner, connecting it to the isoflurane setup for the MR room, connecting the coil, and the DBS leads to the electrodes was all done within a few minutes as well. When planning experiments, one must take in account the extra time setting up and cleaning will take; often clinical MRI sites will not allow equipment for animal experiments to be left on site, and all surfaces and rooms that have been in contact with animals will need thorough cleaning after the experiments.

2.6.6. MRI acquisition and simultaneous DBS
MR images were acquired on a 3.0 T Philips Ingenia MR system.

Fig. 5. Placement of the custom-made restrainer and DBS-MRI setup in a clinical scanner. 1- custom-made connector box connecting the setup’s MRI coil to the scanner’s interface on the scanner bed, 2- restrainer (containing an albino rat) and DBS MRI setup placed on scanner bed, secured between sandbags to minimize movement artifacts, 3- carbon fiber leads running from the DBS electrodes to the patch panel in the scanner room’s wall, 4- tubing for oxygen and isoflurane supply.
imaging (EPI) with interleaved slice acquisition, EPI factor functional MR image acquisition (multishot spin-echo (SE) echo-planar maintenance dose (1.8 % = 1000 ms, TE = 45 ms, dynamic acquisition time = 5000 ms, voxel size = 0.55 × 0.55 × 1.5 mm, matrix size = 64 × 57, 12 slices) was started when the respiration rate of the animal passed 100 breaths per minute. During fMRI acquisition, bilateral DBS (a biphasic, constant current, 80 μs pulse width at 130 Hz, 300 μA stimulation) was delivered to the animals. The stimulation protocol consisted of 20 s of DBS (which equals 4 volumes), followed by 40 s of no stimulation (8 volumes), repeated 10 times. Preceding the 10 repetitions, 8 dummy scans and 40 s of baseline recordings were obtained. Following the fMRI acquisition during which DBS was delivered, the animals again received isoflurane anesthetics (3%, 1:1 air in oxygen at 0.3 L/min) and a T2-weighted anatomical image was obtained (multishot turbo spin echo (TSE) sequence, TSE factor = 8, TR = 4000 ms, TE = 56 ms, voxel size = 0.2 × 0.2 × 1.5 mm, matrix size = 176 × 168, 12 slices). The restrainer setup was then removed from the scanner. While still under anesthetics, the DBS electrodes were connected to a stimulus generator (WPI A365, World Precision Instruments, Berlin, Germany) and electrical lesions were made for determining the electrode tip location during subsequent histology (500 μA direct current for 20 s). The animals were euthanized by terminal cardiac puncture after which the brains were removed, flash frozen, and stored at -80 degrees Celsius for further processing.

2.6.7. Movement parameter analysis

Anatomical and EPI data were converted from DICOM to NIfTI format, using the MRicon/dcm2nii tool (Chris Rorden, 4August2017 (Debian) 64-bit BSD License). Imagej (version 1.50d, National Institutes of Health, Bethesda, Maryland, USA) was used to modify the voxel sizes to ten times their original size to better accommodate the processing algorithms of the FMRIB Software Library (FSL, Analysis Group, FMRIB, Oxford, United Kingdom). FMRIB’s Linear Image Registration Tool (MCFLIRT), with a 6 degrees of freedom (DOF) transformation was used for motion correction of the fMRI time series, using a hand-picked reference volume in the middle of the time series. The motion correction parameters for the left – right, inferior – superior, and anterior – posterior directions were saved for later inspection (trained animals n = 8, untrained animals n = 8).

Motion correction parameters were graphically displayed for visual inspection per direction. For each animal, the absolute, cumulative amount of motion correction needed for the entirety of the fMRI sequence acquisition (10 min) was calculated, and with an independent samples t-test the difference between the amount of correction needed in the untrained versus the trained group was analyzed for each direction.

2.6.8. DBS-induced brain activation analysis

To investigate DBS-induced brain activation, all anatomical and BOLD images of 6 animals were prepared for processing by extracting the brain segment using the FMRIB’s Brain Extraction Tool (FSL/BET). An anatomical template was custom-made, by registering the individual brain-extracted anatomical images to one representative anatomical image and taking the median of those images. The BOLD EPI images of each of the animals were registered to their corresponding anatomical images and non-linearly re-sampled to the FMRIB’s standard Talairach space (using FMRIB’s Linear Image Registration Tool (FLIRT) and FMRIB’s Nonlinear Image Registration Tool (FNIRT), respectively) and carefully checked. The individual anatomical images were then registered linearly to the custom template, after which the resulting transformation matrix was applied to the corresponding functional data.

The FMRIB Expert Analysis Tool (FEAT) was used to apply a general linear model (GLM) to the functional data of the six animals. First, the functional data was smoothed (FWHM 10 mm), high-pass filtered (cutoff 90 s), and FILM pre-whitening was applied. The implemented GLM was a block design modeling stimulation ON (4 volumes during DBS) and stimulation OFF (8 volumes during DBS), repeated 10 times, and preceded by a base line period of 8 volumes. A double-gamma HRF and temporal derivative were added onto the model. Group analyses was subsequently performed by using FMRIB’s Local Analysis of Mixed Effects (FLAME 1 – 2). The statistical significance was set to \( P < 0.05 \) and cluster correction was applied. DBS-induced responses are expressed in z-score units and overlaid as a heat-map on the custom template.

Furthermore, to assess the DBS effect in the ventral striatum, we expressed induced activation as percentage signal change in the nucleus accumbens, as region of interest (ROI). An MRI rat brain atlas was registered to the custom-made template of this study, and an ROI-mask of the nucleus accumbens was made. With use of the featquery – FEAT results interrogation tool (FMRIB Software Library 6.0) the time-series of the ROI was extracted from each of the 6 animals’ functional data. For each animal, the signal intensity during the DBS ON periods were averaged, and of the DBS OFF periods all the second halves were averaged (omitting the first 4 volumes directly after each DBS ON period), as mean signal intensity during DBS ON and DBS OFF, respectively. The 8 volumes preceding the first DBS ON period were averaged as the signal intensity during baseline. The percentage signal change for DBS ON and DBS OFF were subsequently calculated for each animal, and a paired samples t-test was performed to analyze the difference in percentage signal change between the DBS OFF and DBS ON periods in the ROI, using the open-source statistics software JASP (JASP Team, 2020, version 0.14.0.0).

3. Results

3.1. Electrodes

Visual inspection of the T2-weighted images of the custom-made, two-channel electrodes of different materials, indicates that electrodes made of silver induce smaller MRI artifacts than electrodes made of the commonly used platinum/iridium (Fig. 1).

3.2. cFOS

Nine rats with bilateral electrodes in the dVS were stimulated unilaterally (Fig. 6A&B). A paired samples t-test of the relative cFOS density in the areas around the electrode tips, showed a significant difference between the two hemispheres, with a higher relative cFOS density in the hemisphere with DBS ON (\( M = 0.2564, SD = 0.05923 \)) compared to the hemisphere with DBS OFF (\( M = 0.1969, SD = 0.05422 \)) (\( t(8) = 4.387, p < 0.01 \)) (Fig. 6C).

3.3. Corticosterone

The effectiveness of the restrainer training was assessed by analyzing blood plasma corticosterone levels, from samples taken before and during the restrainer session on day 1 and day 6 of training (n = 14)
No significant differences were found when comparing the plasma corticosterone levels (ng/mL) on day 1 with day 6, looking at the samples taken before restraining (day 1: \( M = 106.42, SD = 56.55 \); day 6: \( M = 96.39, SD = 66.39 \); **Fig. 7A**), looking at the samples taken at the beginning of restraining (day 1: \( M = 112.06, SD = 113.53 \); day 6: \( M = 75.00, SD = 57.66 \); **Fig. 7B**), and looking at the samples taken at the end of restraining (day 1: \( M = 181.30, SD = 87.44 \); day 6: \( M = 209.30, SD = 176.61 \); **Fig. 7C**).

When looking at the difference in plasma corticosterone levels at the beginning versus the end of restraining on day 1 compared to day 6 of training (corrected for the level of corticosterone before restraining on that respective day) a significant difference was found, indicating a larger increase of plasma corticosterone during restraining on day 6 (\( M = 203.15, SD = 199.7 \)) compared to day 1 (\( M = 91.66, SD = 160.1 \)) (\( t(13) = -2.204, p < 0.05 \), **Fig. 7E**).
3.4. DBS and MRI

3.4.1. Motion correction

Motion correction parameters for DBS scan session of awake animals that had been subjected to restrainer training (n = 8) and that had not received restrainer training prior to the MRI scan (n = 8) were collected for the left–right, superior–inferior, and anterior–posterior directions for the duration of 10 min, and are shown in Fig. 8A, C and E. The position at time point 0 was set to 0 mm movement in the graphs with all other time points relative to that.

By visual inspection, it can be concluded that for both the trained and the untrained animals, the motion correction parameters do not exceed the acquired voxel dimensions (indicated by grey dotted horizontal lines), and DBS (indicated by grey vertical bars) does not affect the motion correction parameters. Furthermore, no significant difference was found between the total amount of motion correction (defined as the sum of movement corrections applied by the software over the entire 10-minute period) needed to correct for motion during fMRI acquisition between the trained and untrained animals, when comparing the two groups on the corrections for left–right, superior–inferior, and anterior–posterior directions (Fig. 8B, D and F).

3.4.2. Temporal signal-to-noise ratio

The average tSNR within our BOLD fMRI datasets is 15.47 ± 1.90 (n = 16). There was no significant difference in tSNR found between the animals that had received restrainer training prior to going into the scanner (n = 8) and those that had not received training (n = 8) (15.80 ± 2.22 and 15.15 ± 1.60, respectively).

Fig. 8. Motion-correction parameters for fMRI scan sessions in awake rats during DBS in the dorsal part of the ventral striatum (dVS) stay within voxel dimensions and do not differ between animals which received restrainer habituation training (trained) and those that did not (untrained). Panels A, C, and E display motion-correction parameters in left-right (L–R), superior–inferior (S–I), and anterior–posterior (A–P) planes, respectively. fMRI data were analyzed with motion-correction software (MCFLIRT, FMRIB Software Library 6.0). Data were collected during a ten-minute period and are shown as mm displacement from the center of mass. Results from individual animals are shown (green = untrained rats (n = 8), purple = trained rats (n = 8)). Gray vertical bars indicate periods of dVS DBS; no effect of DBS on the movement correction parameters are observed. Voxel dimensions in the L-R, S–I, and A–P directions are 0.55, 0.55, and 1.50 mm, respectively. Voxel dimensions are indicated in each panel by dotted lines. The motion-correction parameters did not exceed voxel dimensions, indicating that the data collected from these animals can be corrected for movement. Panels B, D, and F represent the sum of movement corrections applied by the software over the entire 10-minute period, as individual values (icons) and group averages (bars). These data show that the total, cumulative amount of motion correction applied to the fMRI data by the software, does not differ between the group of trained and untrained animals, in any direction (L–R, S–I, and A–P) (mean ± SD).
3.4.3. DBS-induced activation

To investigate the effect of DBS targeted at the dVS in awake rats, a GLM approach was used to analyze the DBS-induced brain activation in a group of 6 animals. The group analysis shows patterns of activation in the general vicinity of the electrode tips, as well as effects of stimulation outside of that region (Fig. 9). Most specifically, DBS-induced activation can be seen around the stimulation area, the ventral striatum including the nucleus accumbens, in brain areas associated with executive functions such as the caudate putamen, and in prefrontal areas (prelimbic, infralimbic, insular and orbitofrontal cortex), and parts of the primary and cingulate cortices.

To assess the percentage signal change during DBS in the ventral striatum, the nucleus accumbens was chosen as a representative ROI to investigate. When looking at the average percentage signal change during DBS OFF compared to the average percentage signal change during DBS ON (within the group), a significant difference was found using a paired samples t-test (Fig. 10) \( t(5) = -6.509, p = 0.001 \). The results indicate the presence of a higher BOLD signal in the nucleus accumbens during DBS ON periods (M = 1.05 % signal change, SD = 0.345) and a lower BOLD signal during DBS OFF periods (M = -0.59 % signal change, SD = 0.412) compared to baseline.

4. Discussion

We have described the successful combination of bilateral DBS with fMRI measurements in rats that were awake during data acquisition in a clinical 3 T MRI scanner. By using silver as a material for our DBS electrodes, we minimized magnetic susceptibility artifacts in BOLD signal. Stimulation of the ventral striatum affected brain activity near the tip of the DBS electrodes as evidenced by cFOS induction and changes in fMRI BOLD signal, thereby validating successful delivery of electrical current to surrounding brain tissue.

The custom-made surface coil enabled data collection in a 3 T clinical scanner functioning as receiver coil with coil loops encompassing the entire brain, and a two-channel setup enabling parallel imaging. The coil setup combines easy access to the animals’ electrodes and head stage with securing the animals in the restrainer that restricts head movements to an acceptable minimum during MRI scans. Our readouts of stress experienced by the animal and head movements indicated that with our restrainer setup, habituation training prior to MRI acquisition is neither necessary nor beneficial.

Together, the combination of DBS and awake fMR imaging, provides a promising technique to study the effects of DBS on brain activity and functional connectivity in animal models of psychiatric and neurological disorders, without the confounding effects of anesthetics, such as its influence on neurovascular coupling, suppression, and alteration of neural activity and functional connectivity, and changes in cerebral blood flow, compared to awake animals.

Previous fMRI studies in rodent DBS have applied a variety of MRI sequences and electrode materials, but thus far only in anesthetized animals (e.g. Dunn et al., 2009; Young et al., 2011; Lai et al., 2015). Using this approach, Albaugh et al. (2016) reported brain activation induced by stimulation of the ventral striatum. fMRI studies in awake animals (see Ferris et al., 2011, for a review), on the other hand, have

Fig. 9. DBS-induced activation in awake animals. Bilateral DBS applied through silver electrodes targeted at the dorsal part of the ventral striatum (dVS), induces BOLD-activation in awake rats \( (n = 6) \). Notable activation was found in the area around the electrode tips, the ventral striatum including the accumbal area, the caudate putamen, parts of the primary and cingulate cortices, and prefrontal areas (prelimbic, infralimbic, orbitofrontal and insular regions). Electrode locations are indicated by black lines, with arrows indicating the electrode tips. Numbers below the slices indicate relative location to bregma (mm). Color bars denote z score values at a significance threshold of \( p < 0.05 \) (corrected).

Fig. 10. DBS targeted at the dorsal part of the ventral striatum (dVS) induces a significantly higher percentage signal change compared to intermittent periods of no stimulation in the nucleus accumbens of awake rats. A significant difference in percentage signal change in the chosen ROI, the nucleus accumbens (NAc), was found when comparing the DBS ON periods with DBS OFF periods in a group of 6 awake rats. Signal intensity was extracted from the data using featquery (FMRIB Software Library 6.0) with the nucleus accumbens as ROI, from each animal, and the percentage signal change was calculated for DBS ON and the latter half of the DBS OFF periods relative to baseline. A paired sample t-test was performed on the percentages signal change of the DBS ON versus the DBS OFF periods. Mean \( \pm SD, ***, p = 0.001 \).
been developed and applied to study connectivity in neuronal networks (e.g. Chuang and Nasrallah, 2017) or effects of pharmacological and sensory or motivational stimuli (e.g. Febo et al., 2005; Yee et al., 2016). Since DBS in awake rats had not been described yet, we designed a setup that made it possible to perform fMRI acquisition in awake rats.

Our method incorporates a number of technical innovations. First, we pioneered the use of silver electrodes for DBS in rodents, which has not been reported before to our knowledge. Magnetic susceptibility artifacts (distortions or local signal changes due to local magnetic field inhomogeneities) are lower for silver than for platinum/iridium. Our results (Fig. 1) illustrate the higher suitability of silver in this respect. Stimulation using these electrodes readily induces cFOS expression and BOLD activation around the electrodes (Figs. 6 & 9). Why silver has not been used before is unclear – possibly the relative flexibility of the material was considered a potential cause for imprecise electrode placement. However, in our experience this is not the case.

The second innovation is the use of a custom-designed head coil that enabled scanning in a standard clinical 3 T scanner. This coil had to be compatible with the machine’s field strength and connector interface, and allows manual matching and tuning prior to each series of experiments. The combination of our restrainer setup, coil and the 3 T clinical scanner let us effectively conduct our experiments, and was in daily practice straightforward to work with, as setting up the coil was of a plug-and-play nature. The scan settings and sequences were chosen after numerous pilot scans and were optimized and adapted to rat-brain anatomy and hemodynamic responses. After properly placing the coil over the animal’s head and securing it into the setup, it can easily be connected to the interface box. The performance (in terms of temporal signal-to-noise ratio (SNR), and temporal and spatial resolution) is to a large extent determined by the field strength and -homogeneity of the scanner, and the gradient system. The average tSNR of our datasets was 15.5 ± 1.9, which is above the average reported by Welvaert and Rosseel (2013). For a comparison to other small animal DBS-MRI data, temporal and spatial resolution (expressed as TR and voxel size, respectively) may be more useful. The repetition time we used (TR = 4000 ms) was not substantially different from other studies, which combined a 9.4 T scanner and DBS (3000 ms in Young et al., 2011; Lehto et al., 2018; 2260 ms in Dunn et al., 2009), but others reported TRs of 1000 ms or shorter (e.g. Lai et al., 2015; Zhao et al., 2020; Albaugh et al., 2016). Our voxel size of 0.55 × 0.55 × 1.5 is somewhat larger than that what Dunn et al. (2009) used (0.23 × 0.23 × 1.5) or Lehto et al. (2017) (0.55 × 0.55 × 1), but sizes of approximately 0.25 × 0.25 × 1 have been reported by a number of other groups. The performance of the system we describe (in awake rats) is at the lower end of performance compared to dedicated small animal scanners using anesthetized animals. However, we believe that the advantages of our novel method in using awake animals, making preclinical MR imaging experiments more accessible to both animal and clinical researchers, and lowering costs outweigh the disadvantage of lower scan resolution.

The third innovation is creating the possibility to study DBS with MRI in awake animals. In order to do so, a restrainer and head stage-combination was designed to fixate the awake animal in our setup, while also accommodating the custom-designed head coil and leads connecting the DBS electrodes with the stimulator (Figs. 3–5). After an initial phase of getting familiar with this setup, working with it allowed for a smooth and rapid execution of the experiments – an important feat as it is of importance to keep the time the animals are under anesthesia short as possible. With our setup, a wide range of brain MRI experiments becomes available in rodents that are either anesthetized or awake during acquisition.

One of the challenges of performing MRI-scans in awake, conscious animals is preventing movement – in neuroscientific experiments, that is especially motion of the rodents’ heads. Other groups that performed awake animal MRI in combination with a restrainer setup have resorted to acclimating/habituating the animals to being restrained in a series of training sessions prior to the MRI-scan session (King et al., 2005; Febo, 2011; Yee et al., 2016). The motivation to conduct such acclimation procedures is based on findings that suggest that repeated exposure to a stressor results in habituation. More specifically, after multiple exposures, a decrease of overt signs of stress (such as HPA reactivity (i.e. high levels of corticosterone), high heart rate, behavioral struggle during restraint) compared to first-time exposure was reported (Keim and Sigg, 1976; Natelson et al., 1988; review: Grissom and Bhatnagar, 2008). The impact of restraint is not only dictated by the severity of the restraint, but also by its duration, repetition, past stress experience, handling, and intrinsic biological factors (Keim and Sigg, 1976). Previous protocols applied varied strongly (Low et al., 2016) and yielded widely varying results, most notably a lack of habituation to repeated restraint in some cases (e.g. Pitman et al., 1988; Marin et al., 2007). Furthermore, habituation of the corticosterone response does not necessarily imply a reduction or absence of other measures of stress (Low et al., 2016). We examined the effectiveness of restrainer training in our animals, and compared movement of trained and untrained animals during their MRI scan sessions.

Our results demonstrate that basal concentration of blood-plasma corticosterone in animals that receive restrainer training, remained the same across repeated training sessions, indicating that the basal stress-level of the animals is not affected by such habituation sessions (in contrast to previous findings, Keim and Sigg, 1976; Watanabe et al., 1992). We observed no differences between corticosterone levels on days 1 and 6, neither at the start, nor at the end of the restraining period. Instead, we found a mild increase of corticosterone levels in response to the 60-minute restraining. Remarkably, this increase of corticosterone levels during a single training session did not habituate over repeated sessions. On the contrary: comparing the observed increase of corticosterone during day 6 of habituation training with that observed on day 1, shows this increase has become larger over repeated sessions.

Furthermore, looking at the amount of head movements during the actual MRI scan sessions, we found no differences in amount of motion correction needed between trained and untrained animals, indicating that the training of the animals was not beneficial in decreasing head movements and that variable seems to have no relation to our corticosterone data. Importantly, motion correction parameters did never exceed the acquired voxel size during a given 10-minute scan session, and the periods of DBS during those 10 min did not increase head movement. This is also reflected in the tSNR, which was not different between control and trained animals. In other studies, it has been reported that the most dramatic movements occur in the vertical (S–I) direction, possibly as a result of the setup design, and that restrainer training is most effective there (King et al., 2005; Febo 2001). Our data shows no such large movements, in neither the trained nor untrained groups; occasional erratic movements were observed in individual animals, but never exceeding voxel dimensions.

As the restrainer training did not have a significant effect on the head movements during MRI acquisition; did not lead to a habituation of corticosterone increases; and might even result in long-lasting changes in other physiological measures (Low et al., 2016), we conclude that it is not preferred or needed to expose the animals to restrained movement prior to MRI scan sessions. However, different approaches to restrainer training exist, e.g. increasing the duration of restraining gradually over sessions (Febo, 2011; Low et al., 2016), and may lead to more favorable results.

The pilot study in a group of six animals shows that the set-up is applicable to a typical experimental question and provides data of usable quality. The average activation pattern after bilateral dVS stimulation showed bilateral activation of basal-ganglia and cortical areas in the forebrain (Fig. 9). Comparison with published results is challenging, as the only other study of ventral striatal DBS used unilateral stimulation with a more anteromedial placement (Albaugh et al., 2016). The percentage change in BOLD response cannot be compared to that reported by those authors as they used CBV with contrast agent injections. However, the changes we report are within the range of those reported...
by Chao et al. (2014) after unilateral stimulation of the ventro-posterior thalamus. In conclusion, the combination of the custom-made coil and the design of our restrainer enables animal MRI inside a clinical MRI-scanner environment. Clinical scanners, and scanners for humans in general, are much more abundant than pre-clinical small-animal MR sites. Therefore, the possibility to perform animal scanning at clinical scanners is more cost-efficient and requires less effort to set up, compared to outsourcing the experiment or acquiring and maintaining a dedicated small-animal scanner. As the scanner hardware and software will be familiar to trained staff, using our coil and setup combination at Albaugh, D.L., Salzwedel, A., Van Den Berge, N., Gao, W., Stuber, G., Shih, Y.I., 2016. Functional magnetic resonance imaging of deep brain stimulation functional MRI procedures in rats and mice using an MR-compatible tungsten microwire electrode. Magn. Reson. Med. 73 (3), 1246–1251. doi:10.1002/mrm.25239.

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