Microelectrode clusters enable therapeutic deep brain stimulation without noticeable side-effects in a rodent model of Parkinson’s disease

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Background: Deep Brain Stimulation (DBS) is an established treatment for motor symptoms in Parkinson’s disease (PD). However, side effects often limit the usefulness of the treatment.

New method: To mitigate this problem, we developed a novel cluster of ultrathin platinum-iridium microelectrodes (n = 16) embedded in a needle shaped gelatin vehicle. In an established rodent PD-model (6-OHDA unilateral lesion), the clusters were implanted in the subthalamic area for up to 8 weeks. In an open field setting, combinations of microelectrodes yielding therapeutic effects were identified using statistical methods. Immunofluorescence techniques were used for histological assessments of biocompatibility.

Results: In all rats tested (n = 5), we found subsets of 3–4 microelectrodes which, upon stimulation (160 Hz, 60 μs pulse width, 25–40 μA/microelectrode), prompted normal movements without noticeable side effects. Other microelectrode subsets often caused side effects such as rotation, dyskinesia and tremor. The threshold (per microelectrode) to elicit normal movements strongly depended on the number of activated microelectrodes in the selected subset. The histological analysis revealed viable neurons close to the electrode contacts, minor microglial and astrocytic reactions and no major changes in the vasculature, indicating high biocompatibility.

Comparison to existing methods and conclusion: By contrast to the continuous and relatively large stimulation fields produced by existing DBS electrodes, the developed microelectrode cluster enables a fine-tuned granular and individualized microstimulation. This granular type of stimulation pattern provided powerful and specific therapeutic effects, free of noticeable side effects, in a PD animal model.

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ABSTRACT

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1. Introduction

Deep Brain Stimulation (DBS) has been remarkably successful in the treatment of motor symptoms in Parkinson’s disease (PD). It is primarily employed in late stages of the disease when pharmacological treatment no longer provides sufficient symptom alleviation and electrodes are typically placed in the subthalamic nucleus (STN) and surrounding areas. A factor limiting usability is, however, that therapeutic effects are often accompanied by side effects such as spastic muscle contractions (Gorgulho et al., 2009; Tommasi et al., 2008), gaze deviation (Leichnetz, 1981; Shields et al., 2007), gait ataxia (Adams et al., 2011; Fleury et al., 2016), speech disturbances (Krack et al., 2003; Tripoliti et al., 2011; Wertheimer et al., 2014), behavioral changes e.g. aggression (Papuc et al., 2015; Piasecki and Jefferson, 2004) or depression (Combs et al., 2015; Guehl et al., 2006; Strutt et al., 2012), and paresthesia (Kleiner-Fisman et al., 2003; Pahwa et al., 2003). The side effects are assumed to be, at least partly, due to a lack of precision (typically around +/− 1 mm) in positioning the electrode in relation to the target, and/or to inadvertent spread of current to non-targeted areas of the brain (Cagnan et al., 2019; Ineichen et al., 2018). Another problem is that it is not yet clear whether it is stimulation of structures within or outside the STN that produce the therapeutic effects (Blomstedt et al., 2018; Burrows et al., 2012; Landgren et al., 2011), or which sites induce side effects upon stimulation. A further complicating factor is that state-of-the-art DBS electrodes get encapsulated, as indicated by post-mortem histology, which substantially increases the physical, and functional, distance between viable neurons and electrode contacts (Biran et al., 2005; Orlowski et al., 2017; Vedam-Mai et al., 2016). This

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physical barrier likely requires higher stimulation currents to reach therapeutic effects, and therefore enhances the risk for inadvertent spread of current to brain areas inducing side effects. In addition, as the disease progresses, an increase in stimulation current is often required (Hartmann et al., 2015; Limousin and Foltynie, 2019). Recent DBS electrode designs with multiple contacts along the circumference of the shaft of the probe, instead of annular contacts, seem to improve the outcome (Pollo et al., 2014; Rebello et al., 2018; Steigerwald et al., 2018). Nevertheless, the placement of all contacts on the same relatively large physical body provides a significant constrain on the spatial precision that current DBS can achieve.

As a principal alternative, ultrathin flexible microelectrodes have been considered a potential solution for delivering spatially precise stimulation (Lind et al., 2010; Sohal et al., 2016), not least given their favorable biocompatibility (Etemadi et al., 2016; Kohler et al., 2015). However, microelectrode-based DBS is not a panacea as they, in turn, are associated with many challenges, not least since precise implantation of ultrathin electrodes requires structural support, which in itself may cause injuries. Furthermore, during and after implantation, microelectrodes may give rise to micro-punctures of blood vessels and consequent induction of inflammatory reactions at their tips. Another problem, which needs to be addressed, is the size-dependent limitation of delivering sufficient charge from microelectrodes (Cogan et al., 2016; Kuncel and Grill, 2004; Peixoto et al., 2009; Poole-Warren et al., 2010). Considering these challenges and the fact that the precise brain sites to be stimulated are not yet well defined and may even differ between individuals, it remains to be determined whether microelectrode-based DBS is a realistic option.

The aim of the present study was to evaluate the feasibility of microelectrode-based DBS in terms of biocompatibility and obtainable therapeutic effects, and thus mitigate problems associated with currently available DBS electrodes. To this end, we developed a novel electrode design with multiple contacts along the circumference of the shaft of the probe, instead of annular contacts, that improves charge delivery and reduces the risk of puncturing blood vessels. The electrodes were developed and manufactured in house. The final manufacturing steps used in this study are shown in Fig. 1. Platinum-Iridium microwires (Pt90/Ir10), 12.5 µm in diameter (California Fine wires, USA), were attached to a custom-made metal frame and insulated with a 4 µm thick layer of Parylene C (Dichloro-p-cyclophane, Galentis Ltd, Italy) using a Parylene coating machine (Laptop 3000, Para Tech Coating Inc., CA, US). The wires were cut at both ends using 90% high power laser (45% high UV, wavelength 355 nm and energy density 3.3 J/cm2, 50 Hz pulse frequency, target square 100 µm/20 µm, 2 passes) to expose the de-insulated area to high power laser (45% high UV, wavelength 355 nm and energy density 1.89 J/cm2, 50 Hz pulse frequency, target square 100 µm/20 µm, 2 passes) (Etemadi et al., 2016). The wires were cut at both ends using 90% high UV (wavelength 355 nm and energy density 6.6 J/cm2). To enable soldering, the proximal ends of the wires were de-insulated using a butane flame (Portasol Pro-Piezo, USA) and tinned.

Fig. 1. Steps in manufacturing of a microelectrode cluster. (A) A single 12.5 µm thick Pt/Ir wire coated with Parylene C except for the distal 600 µm section. The tip of the wire is equipped with a silicon blob. (B) Four microelectrodes are embedded in a thin gelatin flake, see the dotted line. (C) Stack of four flakes are moulded together into a gelatin probe, the tip was moulded separately.
they were coated with gelatin for 1500 μm from the distal tip (30% gelatin in de-ionized water, 289 Bloom strength, Gelita, Medella Pro 1500, USA) at 50 °C, using a dipping machine (Holmarc Opto-Mechatronics, Dip Coating Unit, HO-TH-01, India). The dipping speed was 3000 μm/sec and the retraction speed was 7000 μm/sec. The microelectrodes were then allowed to dry for 1 hr at room temperature.

Four microelectrodes were aligned in parallel, for a length of approximately 1 cm (measured from the distal edge of the silicon cushions) on a polypropylene co-polymer (Gilbert Curry Industrial Plastics Co Ltd, UK) anti-adherent surface. The alignment of microelectrodes was done under a microscope using a fine paint brush (Pan-duro Hobby AB, Malmö, Sweden) and ethanol (95%). The whole polypropylene sheet along with microelectrodes was subsequently kept on dry ice (CO₂) for 20 min and sprayed with 10% gelatin (101 Bloom strength, Gelita, Medella Pro, 1500, USA) using an air brush (Iwata gravity feed dual action airbrush, Anest Iwata-Medea Inc, Portland, USA). Upon mounting the gelatin flakes containing the microelectrodes was done under a microscope using a fine paint brush (Pan-duro Hobby AB, Malmö, Sweden) and ethanol (95%). The sprayed gelatin froze immediately, thereby fixating the wires.

During the spraying procedure, the proximal parts of the microelectrodes were masked by a sheet of aluminum foil to prevent gelatin deposition at the soldering sites. The gelatin flakes containing the microelectrodes were dried for 1 h at room temperature (Fig. 1B).

After drying, the gelatin flake was cut around the microelectrodes and released from the polypropylene sheet. For identification/tracing of individual flakes, each gelatin flake was color coded at the proximal end. During the spraying procedure, the proximal parts of the microelectrodes were masked by a sheet of aluminum foil to prevent gelatin deposition at the soldering sites. The gelatin flakes containing the microelectrodes were dried for 1 h at room temperature (Fig. 1B).

After drying, the gelatin flake was cut around the microelectrodes and released from the polypropylene sheet. For identification/tracing of individual flakes, each gelatin flake was color coded at the proximal end. Finally, four gelatin flakes were stacked and aligned in a custom-made plexiglass mold (Prototech AB, Helsingborg) for further gelatin deposition. The mold consists of a 9 mm long cylindrical shaft with a diameter of 350 μm, a conical tip at the distal end and a channel for gelatin injection. Upon mounting the gelatin mold, gelatin (30% gelatin, 101 Bloom strength, Gelita, Medella Pro 1500, USA) was injected into the mold, leaving the four flakes fully enclosed by gelatin (Fig. 1C). The gelatin-embedded 16-microelectrode array was dried slowly at 21 °C and 50% relative humidity in a temperature- and humidity-controlled chamber (Rcom, Kungsmaxo 20 digital Incubator). After this, the de-insulated proximal ends were aligned to 16 of the soldering pins of a male Omnetics connector (Plexon, CON/32 m-V/ A8828-001, USA), using a micromanipulator (Luigs & Neumann, Feinmechanik und Elektrotechnik GmbH, Germany), affixed with forceps, and then soldered with tin (SC170, Solder Chemistry, Germany) under a stereo microscope (Olympus, SXz7 0.5–4X, Japan). A 25 μm thick, unsualled pure platinum wire (PT005114, Goodfellow Cambridge Ltd, UK) was soldered to the dedicated ground pin of the Omnetics connector to serve as counter-electrode/reference during stimulation. The connections between the microelectrodes and the connector were sealed with Epoxy (Epoxy Technology, Epotek OG198-54 and 55, USA) and cured to harden with UVB light (Hönlé, Blue Point Eco Led, Germany). Finally, the complete probe was removed from the mold and stored at −20 °C.

2.2. In-vivo studies

2.2.1. Animals

Malmö/Lund animal ethics committee on animal testing approved all the animal procedures related to handling and experiments (Permit number M75-16). Six female Sprague-Dawley rats (Taconic, Denmark), weighing ~200–250 g, were used for the study. All animals received food and water ad libitum and were kept at 21 °C and 65% humidity, with reversed 12 h day and 12 h night cycle. Aseptic conditions were kept throughout all surgical procedures.

2.2.2. Dopamine lesions

Unilateral 6-hydroxydopamine (6-OHDA) lesions were made according to a previously established protocol (Cenci and Lundblad, 2007), under deep anesthesia induced by intraperitoneal injection (i.p.) with 6.3 ml/kg solution of 1 mg/ml Domitor vet (medetomidin hydrochloride; Orion pharma, Turku, Finland) and 50 mg/ml fentanyl (Braun, Aschatenburg, Germany). After shaving and disinfecting their skull with ethanol 70%, rats were mounted into a stereotactic frame with a tooth bar (TB) set to −4.5 mm. The rats received a subcutaneous injection of 0.4–0.6 ml of local anesthetic (xylocaine 2 mg/ml + 1.25 μg/ml, Dentsply Ltd, Surry, UK). During the entire procedure, the rat’s eyes were kept moist by covering them with a net gauze soaked with saline. A midline-incision was made to expose bregma and lambda and a burr hole was drilled at the coordinates AP= −4.4 mm, L= right 1.2 mm from bregma. The rats received two injections of 6-OHDA hydrochloride (3.0 μg/ml dissolved in 0.02% ascorbate saline, Sigma-Aldrich, Sweden) in the area of the medial forebrain bundle at the following coordinates: injection site 1: TB: −5.4, AP: −4.0, ml: 1.2, DV: −7.8; injection site 2: TB: +3.4, AP: −4.0, ml: 0.8, DV: −8.0 (all co-ordinates in mm). After closing the wound, rats received an i.p. injection of antidote (antisedan, atipamezole hydrochloride, 0.5 mg/kg body weight) and a subcutaneous injection of Temgesic (Buprenorphine, 1 mg/kg body weight, s.c. Schering-Plough, Belgium). The rats were left to recover for two weeks after lesioning and were monitored regularly during the recovery period.

2.2.3. Electrode implantation

Anesthesia was induced with a mixture of 30% oxygen, 70% nitrous oxide and 3–4% Isoflurane (Isoba ®vet, Apoteksbolget, Sweden) and maintained with 1.2–2% Isoflurane, using a rodent nose cone (Model 906, Rat anesthesia mask, David Kopf Instruments, California, USA). Body temperature of the rats was maintained at 36–37 °C using a heating pad. The above described procedure of shaving, injecting local anesthesia and keeping the eyes moist (Section 2.2) was repeated during implantation. The head was fixed in a robotic stereotactic frame (Neurostar, Robot Stereotactic instrument, Germany), and the surgery was planned and performed using stereotactic software (Neurostar, Stereodrive 4.0.0, Germany). The head position was measured and calibrated with the robotic software to compensate for size and tilt of the skull.

After removal of connective tissue, a 1.5 × 1.5 mm craniotomy was manually drilled (Kopf Model 1474 High Speed Stereotactic Drill, USA) centred above the coordinates for the implant: AP= −3.6 mm, L= right 2.5 mm with respect to bregma. In addition, 4 stainless steel screws (AgnThos, MCS1x2, Sweden) were anchored in the skull to secure attachment of the probe contact. The probe was implanted medial to the subthalamic nucleus, near zona incerta, in a three-step procedure to a depth of 8 mm from the cortical surface, using the Neurostar robotic system. First, the probe was advanced to a pre-target depth of 5.0 mm with an insertion-speed of 1000 μm/sec. Second, a waiting-period of 4 min followed, allowing the gelatin to swell. Third, the probe was advanced 3 mm further with an insertion-speed of 50 μm/sec into the target, allowing the microwires to spread and form a cluster. A ground-wire was wound around a screw skull and then placed contralaterally on top of the dura mater via a separate burr hole (diameter 0.9 mm). The probe device was stabilized and fixed to the skull bone and anchor screws using dental cement (Relyx ™ Unicem Self-Adhesive Universal Resin Cement). Excessive gelatin surrounding the external part of the probe was removed through rinsing with tempered saline (37 °C) before completely securing the device with dental cement. Finally, animals received a subcutaneous analgesic injection of Temgesic (buprenorphine 1 mg/kg body weight, s.c.) after which anesthesia was discontinued. The rats were left to recover for approximately 1 week before starting experiments.

2.2.4. Behavioral tests

As previously shown, a unilateral 6-OHDA lesion results in rats preferentially turning towards the lesioned side during spontaneous movement (Fornaguera and Schwarting, 2002; Mokrý, 1995). The effects of DBS on motor behavior in 6-OHDA lesioned rats were quantified in an open field, in three sessions per rat (during week 1–2, 3–4 and 4–5 relative to the day of implantation, Fig. 2A). Each stimulation session comprised three trials per animal. The tests were performed during the...
rat’s night-time, in dim light and quiet environment, to promote spontaneous activity and to avoid distraction.

2.2.4.1. Assessment of overall motor behavior. Open field tests were performed to evaluate how stimulation influenced overall motor behavior (Campos et al., 2013; Carvalho et al., 2013). The rat was placed in a custom made square shaped box (80 × 80 cm) with a black background. The behavior of the rats was recorded from above, using a camera (Logitech HD Pro Webcam C920, Logitech Inc) and a head-mounted 3-axes accelerometer (ADXL337, Analog Devices Inc.), both were time-locked to the operation of the electrical stimulator. The movement-data was obtained via video tracking, of the head and body, using in-house developed software in Matlab (MATLAB, 2019, Natick, Massachusetts: The MathWorks Inc., 2019) and analyzed offline. Two LEDs (red facing neck and blue facing nose) were mounted onto the accelerometer that in turn was attached to the rat’s head, allowing detection of the head position and direction. The top of the head was defined as the midpoint between the blue and red LEDs. The (white) body could easily be detected due to its size and high contrast against the dark background. The displacement during a stimulation trial was defined as the total displacement of the head after stimulation-onset and used as a measure of the therapeutic effect. A large displacement score was consistently associated with the animals moving freely without noticeable preference to left or right (e.g., Fig. 4 C), i.e. reversing the symptoms induced by the unilateral 6-OHDA lesion. The most prominent side-effect was an increased preference towards the lesioned side during stimulation (e.g., Fig. 4 B).

2.2.5. In-vivo Impedance measurement

To assess the quality and integrity of the implanted electrodes, the in-vivo impedance was measured before and after each stimulation session. In-vivo impedance (with respect to the ground-wire) of each electrode was measured at 1 kHz using a portable NanoZ™ Impedance tester (NanoZ, v1.4.0, White Matter, LLC, Seattle, Washington, USA).

2.2.6. Deep brain stimulation protocols

The general protocol followed during the 5-week testing period is outlined in Fig. 2A. The stimulation commenced about 1 week post-implantation and was performed via a 16-channel programmable constant-current PlexStim stimulator system (Plexon Inc., Texas, USA) that was connected to the Omnetics head-contact through a commutator, allowing the animal to move around freely during the experiment. Trains of biphasic, charge balanced square pulses of 2 × 60 µs (first phase negative, second phase positive) pulse width and 160 Hz frequency were delivered through the implanted microelectrodes (n = 71) are shown. ** p < 0.01 (Friedman test with Dunn-Sidak post hoc test).

phase negative, second phase positive) pulse width and 160 Hz frequency were delivered through the implanted microelectrodes (Koivuniemi and Otto, 2011). Stimulation was always made against ground. Each stimulation-session lasted up to 3 h, depending on the specific protocol that was being carried out (see below). All stimulation-experiments were controlled with in-house software written in Matlab (MATLAB, 2019a, Natick, Massachusetts: The MathWorks Inc., 2019), enabling systematic experimental procedures and logging of observations, as well as video and accelerometer recordings in a time-stamped manner for objective quantitative analysis.

2.2.6.1. Stimulation protocols used in open field experiments. Initially, all electrodes were stimulated simultaneously, with increasing strength (steps of 2 µA), starting at 10 µA/electrode, until a motor response was detected. The threshold current was defined as the lowest stimulation current resulting in a clear observable motor effect. As an upper limit when finding the threshold, we used the current that would theoretically cause a surface charge density of 30 µC/cm² (Kuncel and Grill, 2004;
This maximum allowed current (per micro electrode) was 98 μA given the calculated area of the deinsulated electrode site and the duration of the stimulation pulse. We next addressed the problem of how to determine which electrode combinations give rise to therapy-like effects upon stimulation. In these experiments, the stimulation current was kept fixed at 1.1 times the threshold current. Given the large number of combinations of electrodes which can be selected from 16 electrodes, it was unrealistic to test all possible combinations. For example, the number of different subgroups consisting of 8 electrodes which can be selected from a total of 16 electrodes, is 12870. We therefore developed a time-efficient procedure to identify the four electrodes that showed to contribute most to the induction of displacement of the animal in the open field test. In the search strategy, 26 electrode combinations including 8 electrodes were pre-defined in a manner such that each of the 16 electrodes was included in a combination an equal number of times (n = 13) (Fig. 2B). Each of the combinations was tested three times in each session (Sessions 1–3, Fig. 2A). Head movements were monitored in real time using a head mounted 3D accelerometer and by tracking the two LEDs on the head (see Section 2.4.1). Each stimulation trial commenced automatically when the accelerometer had not detected a movement for a continuous period of at least 1 s. This motion-based triggering of stimulation was implemented to reduce the overlap between spontaneous and stimulation-induced movements. Ten seconds after onset, stimulation was automatically discontinued, and the program advanced to the next trial. The total displacement of the head obtained in each test was obtained using video tracking of the LEDs. A displacement-score for a given electrode, in each session, was then defined as the average displacement of all trials of the session in which the electrode was involved during stimulation. Based on these scores, the 4 electrodes reaching the highest score were identified.

As a final step in each session 1–3 (Fig. 2A), we assessed the effect of increasing the number of used microelectrodes on the motor response threshold. Initially only the highest ranked electrode was used, and current was ramped up in steps of 2 μA from an initial level of 10 μA. This was done until a clear therapeutic motor effect, without observable side-effects, was achieved. The initial current was then lowered to 10 μA, and the second-highest ranked electrode was added, and the current was ramped up again until a clear motor behavior was elicited, and so forth. The whole procedure was carried out once per session.

2.3. Histology

2.3.1. Perfusion and tissue preparation

Perfusion of the rats was performed 6–8 weeks post implantation. In brief, rats were injected (i.p.) with an overdose of pentobarbital (150–200 mg/kg, Apoteket Product and Laboratories AB, Stockholm, Sweden). After ensuring there was no response to painful stimulus, rats were transcardially perfused with room tempered (RT; 22 ± 1 °C) physiological saline (0.9%) until clear from blood (~80–100 ml), followed by ~320 ml ice cold 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer at pH 7.4, and ~80 ml of 0.9% saline. The electrodes

Fig. 4. Movement trajectory elicited by three different subsets of microelectrodes, each including 8 microelectrodes. Three trials from the same session, in one rat. Dots in right corner represents the 16-microelectrode cluster, stimulated microelectrodes in pink and non-stimulated microelectrodes in blue. The white dashed square indicates the borders of the open field arena. Green and red dots indicate start and stop positions, respectively. (A) A sample subset of eight electrodes which evoked no displacement. (B) A sample subset causing rotation to the right (with respect to the animal trajectory) (C) A sample subset causing a movement without bias towards left or right.
were carefully explanted, and brains were dissected and cryoprotected in 20% sucrose solution for ~45 hrs. Subsequently, the brains were snap frozen in 2-methylbutane on dry ice and stored at −80 °C until sectioning. Horizontal sections of 16 µm thickness were cut using a cryostat (Microm HM 560, Microm GmbH, Walldorf, Germany), mounted on glass slides (Super Frost plus, Menzel-Glaser, Germany), and stored at −20 °C until immunohistochemical staining.

2.3.2. Histological assessment

2.3.2.1. Tyrosine hydroxylase staining. Tyrosine hydroxylase staining was done to determine the extent of the 6-OHDA lesion. Briefly, sections were rinsed in phosphate buffered saline (PBS, 3 × 5 min), treated with hydrogen peroxide (0.3% H2O2, for 15 min) to block endogenous peroxidase, followed by rinsing in PBS (3 × 5 min), all at room temperature (RT). To prevent unspecific binding, sections were blocked with 5% goat serum in 0.25% Triton X in PBS for 60 min, and subsequently incubated with rabbit anti-tyrosine hydroxylase antibody (1:1000, #P40101-1520, Pel-Freeze Biologicals, Arkansas, USA) in the blocking solution at 4 °C overnight. The following day, sections were rinsed in PBS (3 × 5 min) and incubated with biotinylated goat-anti-rabbit in blocking solution (1:200, #BA-1000, Vector Laboratories, Burlingame, USA) for 60 min at RT. After rinsing in PBS (3 × 5 min), sections were incubated with VECTASTAIN Elite ABC Reagent in PBS (avidin-biotin-HRP complex; Vectorstain™ ABC Elite kit, #PK-6100, Vector Laboratories, Burlingam, CA) at RT for 60 min. This was followed by detection of antibody-antigen binding by applying 3,3′-dia-mino benzidine (DAB Peroxidase substrate kit, #SK-4100, Vector Laboratories, Burlingame, USA) on the sections in the fume hood (incubation ~5 min). Finally, sections were rinsed in distilled water (5 min) and cover-slipped with polyvinyl alcohol mounting media with 1,4-diazabicyclo(2.2.2)octane (PVA-DABCO, Merck/Sigma Aldrich, Sweden).

Low magnification digital pictures of the stained sections were captured using an Olympus camera (SDF PLAPO 1X PF, Olympus corporation) for TH staining quantification. Optical density of TH fibers was measured according to a previously described method (Burke et al., 1990) using NIH ImageJ software. In short, the corpus callosum (where TH-positive fibers are absent) was used to estimate the TH background level and this was subtracted before comparing the differences between lesioned and non-lesioned sides. The data is expressed as the percentage of TH loss on the lesioned side compared to the non-lesioned side.

2.3.2.2. Neuronal, glial and blood vessel staining. An additional immunohistological analysis was performed to evaluate changes in neuronal and blood vessel density, as well as microglial and astrocytic reactions in response to the implanted electrode cluster. In brief, frozen sections were thawed at RT for 30 min, rehydrated in PBS (3 × 10 min) followed by blocking in 5% goat serum in 0.25% Triton X-100 in PBS for 60 min to prevent unspecific binding. Sections were subsequently incubated with primary antibodies overnight at RT. The following antibodies were used: i) rabbit anti-neuronal nuclei (NeuN expressed in neuronal nuclei, 1:500, #Rb 104225, Abcam, USA), ii) mouse anti-RECA (rat endothelial cell antibody against endothelial cells lining blood vessel, 1:1000, #MA970R, Bio-Rad/ Serotec), iii) mouse anti-Cd68/ED1 (marker for activated microglial cells, 1:250, #MA341R,Bio-Rad/ Serotec, UK), and iv) rabbit anti-glial fibrillary acid protein (GFAP, astrocytic cytoskeletal protein; 1:5000, #Z0334, Dako, Denmark). The following day, slides were rinsed in PBS (3 × 10 min) followed by incubation with secondary antibodies i) goat anti-rabbit Alexa 594 (1:1000, Invitrogen, USA), ii) goat anti-mouse Alexa 488 (1:500, Invitrogen, USA) and DAPI (4′, 6-diamidino-2-phenylindole, 1:1000, Invitrogen, USA) in the dark for 2hrs at RT. Finally, the slides were rinsed with PBS (3 × 10 min) and cover-slipped with PVA-DABCO (Merck/Sigma Aldrich, Sweden), and stored at 4 °C.

2.3.2.3. Image acquisition and analysis. To evaluate the effects of implanted electrodes on the brain tissue, digital images were captured using a Nikon Eclipse 80i microscope connected to a Nikon DS-R1 camera. The tissue was screened in sections along the implantation track and the final depth observed (using the ED1 response as a guide). After this, images in the depth-range (100–500 µm from the endpoint of the array) were captured and analyzed. The sections were photographed under a 10x objective, with the same gain and contrast for all markers, the exposure time was set specifically for each marker but remained the same for all images. NIS-Elements BR software 3.05 (NIS-Elements, Nikon Instruments, Japan) was used for image acquisition and analysis. The electrode arrays were identified under 10x and subsequently localized in images captured with 2x magnification to get an overall picture of their location in the brain. To estimate the spread of the electrode clusters the distance between the wires furthest away from each other was measured. For quantification of the ED1 activation in response to implanted electrodes, the diameter of the ED1-stained area was measured around single wires in the sections with the maximal wire spread at the relevant depth range.

2.4. Statistical analysis

Out of 6 rats, one rat was excluded from the analysis due to incorrect positioning of the probe. Impedance measurements from microwires with impedances higher than 2 MΩ, indicating absence of proper contact, were excluded from statistical analysis. Statistical analysis of impedance data was performed using Matlab (MATLAB, 2019, Natick, Massachusetts: The MathWorks Inc., 2019). Impedance changes between sessions were analyzed using a Friedman test, followed by a Dunn-Sidak multiple comparisons post hoc test. Impedance changes within sessions (before vs. after stimulation) were analyzed using a Wilcoxon matched-pairs signed-rank test. Single regression analysis was used for analysis of the relationship between the number of electrodes and stimulation thresholds per electrode, using GraphPad Prism 8.1.2 (GraphPad Inc., USA).

3. Results

3.1. Characterization of implanted probe

In the present study, a novel multichannel cluster probe for high-precision stimulation of neural tissue was developed (Fig. 1) and tested in vivo. As shown in the subsequent histological evaluation, the average spread of the microelectrodes in the cluster was 674 ± 125 µm (mean ± SD; n = 5). The probes were implanted in the subthalamic area near zona incerta in five 6-OHDA treated rats showing clear unilateral motor impairment in the open field test. To check the integrity of the microelectrodes, the impedance (at 1 kHz) was measured directly before and after each stimulation session. The impedance measured before stimulation sessions increased significantly from session 1 to session 2 (P < 0.01, n = 71), but was unchanged between sessions 2 and 3 (Fig. 3). Impedance was consistently lower when measured after stimulation sessions as compared to before stimulation sessions (session 1, P < 0.001, n = 62; session 2, P < 0.001, n = 70; session 3, P < 0.001, n = 79). Variation in the number of included microelectrodes (n) was due to occasional mechanical problems with the adapter to the impedance-measurement device.

3.2. Effects of deep brain stimulation

The mean base threshold current for eliciting motor responses in the open field test when including all electrodes in the probe was 18.4 ± 4.8 µA (mean ± SD) per electrode, i.e. on average less than 20% of the maximum allowed current of 98 µA. With a 10% addition to the threshold current, the current used during stimulation with combinations of eight electrodes was 20.2 ± 5.3 µA (mean ± SD) per electrode.
In each rat, the motor effects of 26 combinations of eight electrodes were tested (Fig. 2A). Each combination was tested 3 times per session thus yielding 78 trials per session. In general, the behavioral responses differed between different combinations, although some combinations evoked rather similar behaviors. Thus, a multitude of behavioral effects could be elicited using different combinations of electrodes, spanning from no effect on movement (Fig. 4A), increased rotation (Fig. 4B), improvements in locomotion (Fig. 4C) but also initiation of grooming, and rearing. Side effects such as tremor or dyskinesia were observed during stimulation via some microelectrode combinations, but not quantified. Importantly, similar motor behaviors were elicited in the same rat when using the same combination of electrodes during different sessions of the experiments (Fig. 5). For example, the combination of electrodes which evoked locomotor activity in one session, also evoked locomotor activity in subsequent sessions and the combination of electrodes which evoked rotation, or no locomotor activity produced similar results during subsequent trials. These findings indicate that a high degree of stimulation specificity can indeed be achieved by selecting appropriate subgroups of electrodes from the implanted clusters.

We proceeded by analyzing the effects of the four highest scoring electrodes. As can be seen in Fig. 6, the threshold current per electrode was reduced progressively with the number of electrodes used (single regression analysis, \( P < 0.001, R^2 = 0.80 \)). In all rats, the currents were well below the maximum safe current-level of 98 \( \mu A \) (see Methods). Furthermore, in all rats tested, we found subsets of 3–4 microelectrodes which, upon stimulation (25–40 \( \mu A \)/microelectrode), prompted normal movements without noticeable side effects (Fig. 6A and C).

### 3.3. Histological assessments

#### 3.3.1. Lesion verification and probe location

TH-staining of the striatum was used to examine the extent of degeneration of dopaminergic innervation and thus to evaluate the 6-OHDA lesioning. In four of the five animals, the relative optical intensity of the lesioned hemisphere was reduced by 97 ± 6% (mean ± SD) compared to the non-lesioned side (Fig. 7). However, in the fifth animal, the TH staining was only reduced by 73%. The electrode array locations were examined and identified medially to the STN near the zona incerta in all five animals.

#### 3.3.2. Neuronal, glial and blood vessel staining

In all animals, neurons were found in close proximity (<10 \( \mu m \)) to individual electrodes in the cluster array (Fig. 8A, B), at the electrophysiologically relevant depth-range of stimulation. A diffuse astrocytic response, examined after staining for GFAP, was found near the implantation, primarily originating from around the wire locations (Fig. 8C). However, no obvious signs of encapsulation or glial thickening could be detected around the wires in any of the animals. The RECA staining (antigen expressed by endothelial cells lining blood vessels)/blood vessel density was on the contrary not obviously changed throughout this area (Fig. 8D) or compared to the surrounding tissue (Supplementary Fig. 1), apart from a slight decline at the location of the individual wires where there occasionally is a small void in the tissue after explantation of the wires. The radius of activated microglia (ED1 immunoreactivity) around the single electrode wires was quantified in the sections with the maximal wire spread inside the electrophysiologically relevant depth range for each animal and was found to extend...
8.9 ± 5.8 µm (Fig. 8B) around the individual wires.

4. Discussion

The present work shows that, using our novel electrode design and stimulation protocol, significant beneficial effects without noticeable side effects can be obtained with relatively low stimulation current, by selecting therapeutically effective microelectrodes from an implanted cluster (Fig. 6). Moreover, glial reactions around the implanted electrodes were rather small and there were no signs of changes in blood vessel density around the wires in the target area, indicating a high degree of biocompatibility.

4.1. On the mechanisms of side effects

It is a common notion that side effects during DBS are due to inadvertent spread of stimulation current outside the therapeutic target area (Ineichen et al., 2018; Shukla et al., 2017). However, apart from that side effects often can be elicited by increasing the stimulation strength and thus affecting neurons at a distance, little evidence has been presented to show that this is the main mechanisms underlying the frequent occurrence of side effects during conventional DBS treatment. In the present study, side effects at low stimulation intensities were consistently associated with stimulation via specific electrodes and thus particular groups of neurons/ pathways in the brain. This finding is consistent with the notion that side effects, such as dyskinesia or tremor, arise when abnormal activity in specific groups of neurons or pathways is induced (Cenci et al., 2018; Cenci and Konradi, 2010; Fieblinger and Cenci, 2015). The inherent feature of the present technique, that the spatial stimulation pattern can be individually tailored, excluding inappropriate locations from stimulation, thus appears to be key to obtain useful therapeutic effects.

4.2. On the capacity of microelectrodes to produce therapeutic effects

Conventional DBS electrodes for human use have much larger contact surfaces than the present microelectrodes and can therefore be used within safety limits for stimulation of larger volumes of brain tissue. However, considering the complexity of brain networks, the diffuse and spatially imprecise nature of such stimulation also results in limited possibilities to avoid concomitant stimulation of neuronal networks eliciting side effects. While much higher stimulation specificity can be reached when using single microelectrodes, a more limited stimulation zone around each microelectrode can be achieved without exceeding the established safety level for surface charge density of 30 µC/cm² (Kuncel and Grill, 2004; McCreery et al., 1990). Thus, a more precise placement is required. Hitting the beneficial sites during surgery can be problematic, since the brain targets and the underlying therapeutic mechanisms are not fully identified (Lozano et al., 2019). There may also be small anatomical differences between individuals. The technique developed here reduces some of these problems by allowing a cluster of microelectrodes to spread out in the target area. However, to be useful in humans, having approximately a 1000-fold larger brain volume than a rat (Defelipe, 2011; Herculano-Houzel, 2009), the problem of limited capacity for charge delivery from individual microelectrodes needs to be addressed. The present finding that significantly lower stimulation
thresholds per microelectrode were obtained by stimulating a selection of microelectrodes instead of using an individual microelectrode, might provide at least a partial solution to this problem. Furthermore, an upscaled probe, comprising a greater number of microelectrodes and/or microelectrodes with larger contact surfaces, as well as a more extensive spread of the microelectrodes, is feasible. While a larger number of microelectrodes would provide improved selection and thus a higher degree of specificity, the time to find useful combinations of therapeutic microelectrodes would likely increase. Fortunately, the present study in degree of specificity, the time to find useful combinations of therapeutic microelectrodes would provide improved selection and thus a higher spread of the microelectrodes, is feasible. While a larger number of microelectrodes with larger contact surfaces, as well as a more extensive spread of the microelectrodes, is feasible. While a larger number of microelectrodes would provide improved selection and thus a higher degree of specificity, the time to find useful combinations of therapeutic microelectrodes would likely increase. Fortunately, the present study indicates that many combinations of microelectrodes can produce useful therapeutic effects. Moreover, the statistical search method introduced here using subgroups of 8 electrodes, required a rather limited number of tests to define useful combinations. In view of the rapid onset/offset of stimulation effects and thus short evaluation time for each test, the time to find a suitable combination of microelectrodes may not be a significant problem in a clinical setting. It may also be worth noting that the developed method permits tuning and further optimization of the stimulation parameters, also after an acceptable result has been obtained, something which can be performed during the ongoing treatment.

4.3. Biocompatibility aspects and stability of microelectrode performance

It is a common observation that stiff, needle-like microelectrodes, used for example to locate proper brain sites for subsequent implantation of electrodes for DBS, relatively often cause bleeding (Ben-Haim et al., 2009; Binder et al., 2003; Xiaowu et al., 2010). An increased number of such microelectrodes would, consequently, increase the risk for bleeding. Moreover, stiff microelectrodes usually give rise to a “kill zone” with loss of neurons and substantial glial reactions due to micromotions in the brain (Biran et al., 2005; Gilletti and Muthuswamy, 2006; Kohler et al., 2015; Thelin et al., 2011). This, in turn, increases the current intensities necessary to activate healthy neurons. Thus, an important requirement to be able to use microelectrodes for DBS, includes a high degree of biocompatibility. Consequently, we used ultra-thin and flexible microelectrodes, which have been shown to be more biocompatible than stiff electrodes (Lee et al., 2017; Sohal et al., 2016), and embedded them in a needle shaped gelatin vehicle to allow implantation. Gelatin has been shown to mitigate microglia activation, reduce the loss of neurons near the microelectrodes (Kohler et al., 2015; Lind et al., 2010), and promote healing of the blood-brain-barrier after insertion (Kumosa et al., 2018). As an extra precaution, rounded silicon blobs were attached at the distal ends of the individual microelectrodes, to reduce the risk of puncturing blood vessels during and after implantation. The findings of essentially normal density of vascular vessels in the cluster area, viable neurons at micrometer distances from the present microelectrodes, minor glial reactions, and stable impedance and stimulation thresholds, indicate a high degree of biocompatibility. These findings also confirm that the tissue is not damaged when proper stimulation parameters are used (Mccreery et al., 1990; Shannon, 1992).

4.4. Limitations of the study

The absence of a severe tissue response to the implanted electrodes, as well as the stability of effects observed between stimulation sessions, are promising signs for long-term stability. However, the robustness of effects over longer stimulation periods remains to be assessed.

CRediT authorship contribution statement

MM: conceiving the study, contributing to experimental work, data analysis, and writing the manuscript. NL: contributing to the experimental work and manuscript writing. HB: conceiving the study and contributing to writing the manuscript. PT: responsible for data analysis, and contributing to the manuscript writing. LMEP: responsible for histology data analysis, and contributing to the writing the manuscript. JT: contributing to the experimental work and manuscript writing. JS: conceiving the study and writing the manuscript.

Declaration of Competing Interest

Jens Schouenborg is the inventor of a pending and issued patents on

![Immunofluorescent staining of tissue reactions around the microelectrode cluster array.](image)
flexible electrodes embedded in dissolvable matrix material and methods of their use and is a cofounder of Neuronano AB, Sweden that owns the patents. Mohsin Mohammed is a co-inventor of a pending patent application on microelectrode based deep brain stimulation. Hjalmar Bjartmarz is a co-inventor on pending patents on methods for microelectrode stimulation. The other authors have no competing financial interests. There has been no significant financial support for this work that could have influenced its outcome.

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Appendix A: Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neumeth.2021.109399.

References

Adams, C., Keep, M., Martin, K., McVicker, J., Kumar, R., 2011. Acute induction of leucopenia resistant frequently gait upon subthalamic nucleus microelectrode implantation. Park. Relat. Disord. 17, 488–490. https://doi.org/10.1016/j.parkreldis.2011.02.014.

Ber-Haim, S., Asaad, W.F., Gale, J.T., Eskandar, E.N., 2009. Risk factors for hemorrhage during microelectrode-guided deep brain stimulation and the introduction of an improved microelectrode design. Neurosurgery 64, 753–754. https://doi.org/10.1227/01.NEU.0000319777.2403.49.

Binder, D.K., Rau, G., Starr, P.A., 2003. Hemorrhagic complications of microelectrode-guided deep brain stimulation. Stereo. Funct. Neurosurg. 80, 28–31. https://doi.org/10.1159/000075156.

Biran, R., Martin, D.C., Tresco, P.A., 2005. Neuronal cell loss accompanies the brain tissue response to chronically implanted silicon microelectrode arrays. Exp. Neurol. 195, 115–126.

Blomstedt, P., Stennert Persson, R., Hariz, G.M., Linder, J., Fredricks, A., Haggstrom, B., Carvalho, M.M., Campos, F.L., Coimbra, B., Pego, J.M., Rodrigues, C., Lima, R., Cagnan, H., Denison, T., McIntyre, C., Brown, P., 2019. Emerging technologies for deep brain stimulation of the subthalamic nucleus in patients with Parkinson’s disease: a meta-analysis. Neurosurgery. Res. 4, 41–49.

Gillette, A., Muthuswamy, J., 2006. Brain micromotion around implants in the rodent somatosensory cortex. J. Neurol. Eng. 3, 189–195. https://doi.org/10.1088/1741-5503/3/3/001.

Gorga, A.A., Shields, D.C., Mallakian, D., Behnke, E., Desalles, A.A., 2009. Stereotactic coordinates associated with facial musculature contraction during high-frequency stimulation of the subthalamic nucleus. J. Neurosurg. 110, 1317–1321. https://doi.org/10.3171/2008.8.JNS08825.

Güelü, D., Ozyer, E., Benazzouz, A., Rougier, A., Tison, F., Machado, S., Grabot, D., Gross, C., Bioulac, B., Barbuda, P., 2006. Side-effects of subthalamic stimulation in Parkinson’s disease: clinical evolution and predictive factors. Eur. J. Neurol. 13, 963–971. https://doi.org/10.1111/j.1468-1331.2006.00405.x.

Harmatz, C.J., Wojtke, L., Vesper, J., Vollkom, J., Gros, S.I., Schnitzler, A., Südmeyer, M., 2015. Long-term evaluation of impedance levels and clinical development in subthalamic deep brain stimulation for Parkinson’s disease. Park. Relat. Disord. 21, 1247–1250. https://doi.org/10.1016/j.parkreldis.2015.07.019.

Hermosillo, H., 2009. Long-term follow up in numbers: a linearly scaled up primate brain. Hum. Neurosci. 3, 31 https://doi.org/ARTN 31.389./neuro.09.031.2009.

Hiwatashi, N., Hirano, S., Mizuta, M., Tateya, I., Kanemaru, S., Nakamura, T., Ito, J., Kawai, K., Suzuki, S., 2015. Biocompatibility and efficacy of collagen/gelatin sponge scaffold with sustained release of basic fibroblast growth factor on vocal fold fibroblasts in 3-dimensional culture. Ann. Otol. Rhinol. Laryngol. 124, 116–125.

Ineichen, C., Shepherd, N.R., Surucu, O., 2018. Understanding the effects and adverse reactions of deep brain stimulation: is it time for a paradigm shift toward a focus on heterogeneous biophysical tissue properties instead of electrode design only? Front. Hum. Neurosci. 12, 468. https://doi.org/10.3389/fnhum.2018.00468.

Kleiner-Fisman, G., Fisman, D.N., Sime, E., Saint-Cyr, J.A., Lozano, A.M., Lang, A.E., 2003. Long-term follow up of bilateral deep brain stimulation of the subthalamic nucleus in patients with advanced Parkinson disease. J. Neurol. Neurosurg. Psychiatry 89, 489–495. https://doi.org/10.1136/jnnp.2013.305489.

Kohler, P., Wolfch., A., Ejerholm, F., Vollkom, J., Schouenborg, J., Linneerier, C.E., 2015. Influence of probe flexibility and gelatin embedding on neuronal density and glial responses to brain implants. PLoS One 10, e0119340. https://doi.org/10.1371/journal.pone.0119340.

Koivunen, A.S., Otto, K.J., 2011. Asymmetric versus symmetric pulses for cortical microstimulation. IEEE Trans. Neural Syst. Rehabil. Eng. 19, 468–476.

Krack, P., Batir, A., Van Blereom, N., Chabardes, S., Fraix, V., Ardon, C., Koudsie, A., Limousin, P.B., Benazzouz, A., LeFauf, J.F., Benabid, A.L., Pollak, P., 2003. Five-year follow-up of bilateral stimulation of the subthalamic nucleus in advanced Parkinson’s disease. N. Engl. J. Med. 349, 1925–1934. https://doi.org/10.1056/NEJMoa035275.

Kumosa, L.S., Zetterberg, V., Schouenborg, J., 2018. Gelatin promotes rapid restoration of vocal fold fibroblasts in 3-dimensional culture. Front. Bioeng. Biomat. 6, 6. https://doi.org/10.3389/fbioe.2018.00006.

Lee, H.C., Ejserholm, F., Gaire, J., Currlin, S., Schouenborg, J., Wallmark, L., Bengtssohn, M., Park, K., Otto, K.J., 2017. Histological evaluation of flexible neural implants: flexibility limit for reducing the tissue response? J. Neural Eng. 14, 36026. https://doi.org/10.1088/1741-2552-14-3-036026.

Limousin, P., Foltynie, T., 2019. Long-term outcomes of deep brain stimulation in patients with advanced Parkinson disease. J. Neurol. Neurosurg. Psychiatry 89, 489–495. https://doi.org/10.1136/jnnp.2013.305489.

M. Mohammed et al.

Chapter 9 9, 25. https://doi.org/10.1002/0471142301.ns0925s41.

Parkin. Dis. 2011, 658956.

Park. Dis. 2011, 658956.
M. Mohammed et al.

Mccreery, D.B., Agnew, W.F., Yuen, T.G.H., Bullara, L., 1990. Charge-density and charge per phase as cofactors in neural injury induced by electrical-stimulation. IEEE Trans. Biomed. Eng. 37, 996–1001. https://doi.org/10.1109/10.92612.

Moky, J., 1995. Experimental models and behavioural tests used in the study of Parkinson’s disease. Physiol. Res 44, 143–150.

Orlowski, D., Michalits, A., Glud, A.N., Korshøj, A.R., Fitting, L.M., Mikkelsen, T.W., Mercanzini, A., Jordan, A., Dransart, A., Sørensen, J.C.H., 2017. Brain tissue reaction to deep brain stimulation—a longitudinal study of DBS in the Goettingen minipig. Neurostimulation 20, 417–423. https://doi.org/10.1111/ner.12576.

Pahwa, R., Wilkinson, S.B., Overman, J., Lyons, K.E., 2003. Bilateral subthalamic stimulation in patients with Parkinson disease: long-term follow up. J. Neurolung. 99, 71–77. https://doi.org/10.3171/jns.2003.99.1.0071.

Papuc, E., Trojanowski, T., Obszanska, K., Stelmasiak, Z., 2015. Aggressive behavior as a rare side effect of subthalamic stimulation in Parkinson disease. Neurocase 21, 220–225. https://doi.org/10.18934/13554794.2014.890729.

Peixoto, N., Jackson, K., Samiyi, R., Minnikanti, S., 2009. Charge storage: stability measures in implantable electrodes. Conf Proc Annu Int Conf IEEE Eng Med Biol Soc IEEE Eng Med Biol Soc Annu Conf, pp. 658–661.

Piasecki, S.D., Jefferson, J.W., 2004. Psychiatric complications of deep brain stimulation. J. Neurosurg. 100. https://doi.org/10.1093/brain/awu102.

Poole-Warren, L., Lovell, N., Baek, S., Green, R., 2010. Development of bioactive conducting polymers for neural interfaces. Expert Rev. Med Devices 7, 35–49.

Rebelo, P., Green, A.L., Aziz, T.Z., Kent, A., Schafer, D., Venkatesan, L., Cheera, B., 2018. Thalamic directional deep brain stimulation for tremor: spend less, get more. Brain Stimul. 11, 600–606. https://doi.org/10.1016/j.brs.2017.12.015.

Shields, D.C., Gorgulho, A., Behneke, E., Malkasian, D., Desalles, A.A., 2007. Contralateral conjugate eye deviation during deep brain stimulation of the subthalamic nucleus. J. Neurosurg. 107, 37–42. https://doi.org/10.3171/NS-07/07/0037.

Shuhla, A.W., Zeilman, P., Fernandez, H., Bajwa, J.A., Mehanna, R., 2017. DBS Programming: an evolving approach for patients with Parkinson’s disease. Park. Relat. Disord. 2017 https://doi.org/10.1016/j.parkreldis.2017.09.015.

Sohal, H.S., Clowry, G.J., Jackson, A., O’Neill, A., Baker, S.N., 2016. Mechanical flexibility reduces the foreign body response to long-term implanted microelectrodes in rabbit cortex. PLoS One 11, e0165606. https://doi.org/10.1371/journal. pone.0165606.

Steigerwald, F., Matthies, C., Volkmann, J., 2018. Directional Deep Brain Stimulation. https://doi.org/10.1007/s13311-018-0667-7.

Strutt, A.M., Simpson, R., Jankovic, J., York, M.K., 2012. Changes in cognitive-emotional and physiological symptoms of depression following STN-DBS for the treatment of Parkinson’s disease. Eur. J. Neurol. 19, 121–127. https://doi.org/10.1111/j.1468-1331.2011.03447.x.

Thelin, J., Jerntell, H., Pouw, E., Garwicz, M., Schouenborg, J., Danielsén, N., Linne, C.E., 2011. Implant size and fixation mode strongly influence tissue reactions in the CNS. PLoS One 6, 10.

Tommasi, G., Krack, P., Frax, V., Le Bas, J.F., Chabardes, S., Benabid, A.L., Pollak, P., 2008. Pyramidal tract side effects induced by deep brain stimulation of the subthalamic nucleus. J. Neurol. Neurosurg. Psychiatry 79, 813–819. https://doi.org/10.1136/jnnp.2007.117507.

Tripoliti, E., Ritzno, L., Martinez-Torres, I., Frost, E., Pinto, S., Polynie, T., Helle, P., Petersen, E., Roughton, M., Hariz, M.I., Limouzin, P., 2011. Effects of subthalamic stimulation on speech of consecutive patients with Parkinson disease. Neurology 76, 80–86. https://doi.org/10.1212/WNL.0b013e318203e780.

Vedam-Mai, V., Rodgers, C., Gureck, A., Vincent, M., Ippolito, G., Elkovaz, A., Yams, A., Foote, K.D., Okun, M.S., 2018. Deep brain stimulation associated gliosis: a post-mortem study. Park. Relat. Disord. 54, 51–55. https://doi.org/10.1016/j. parkreldis.2018.04.009.

Wertheimer, J., Gottuso, A.Y., Nuno, M., Walton, C., Dubois, A., Tuchman, M., Ramaig, L., 2014. The impact of STN deep brain stimulation on speech in individuals with Parkinson’s disease: the patient’s perspective. Park. Relat. Disord. 20, 1065–1070. https://doi.org/10.1016/j.parkreldis.2014.06.010.

Xiaowu, H., Xiufeng, J., Xiaoping, Z., Bin, H., Laixing, W., Yiqun, C., Jinchuan, L., Aiguo, J., Jianmin, L., 2017. Risks of intracranial hemorrhage in patients with Parkinson’s disease receiving deep brain stimulation and ablation. Park. Relat. Disord. 16, 96–100. https://doi.org/10.1016/j.parkreldis.2009.07.013.