



Research report

Wireless stimulation of the subthalamic nucleus with nanoparticles modulates key monoaminergic systems similar to contemporary deep brain stimulation

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ABSTRACT

Background: Deep brain stimulation (DBS) is commonly used to alleviate motor symptoms in several movement disorders. However, the procedure is invasive, and the technology has remained largely stagnant since its inception decades ago. Recently, we have shown that wireless nanoelectrodes may offer an alternative approach to conventional DBS. However, this method is still in its infancy, and more research is required to characterize its potential before it can be considered as an alternative to conventional DBS.

Objectives: Herein, we aimed to investigate the effect of stimulation via magnetoelectric nanoelectrodes on primary neurotransmitter systems that have implications for DBS in movement disorders.

Methods: Mice were injected with either magnetoelectric nanoparticles (MENPs) or magnetostrictive nanoparticles (MSNPs, as a control) in the subthalamic nucleus (STN). Mice then underwent magnetic stimulation, and their motor behavior was assessed in the open field test. In addition, magnetic stimulation was applied before sacrifice and post-mortem brains were processed for immunohistochemistry (IHC) to assess the co-expression of c-Fos with either tyrosine hydroxylase (TH), tryptophan hydroxylase-2 (TPH2) or choline acetyltransferase (ChAT).

Results: Stimulated animals covered longer distances in the open field test when compared to controls. Moreover, we found a significant increase in c-Fos expression in the motor cortex (MC) and paraventricular region of the thalamus (PV-thalamus) after magnetoelectric stimulation. Stimulated animals showed fewer TPH2/c-Fos double-labeled cells in the dorsal raphe nucleus (DRN), as well as TH/c-Fos double-labeled cells in the ventral tegmental area (VTA), but not in the substantia nigra pars compacta (SNc). There was no significant difference in the number of ChAT/ c-Fos double-labeled cells in the pedunculopontine nucleus (PPN).

Conclusions: Magnetoelectric DBS in mice enables selective modulation of deep brain areas and animal behavior. The measured behavioral responses are associated with changes in relevant neurotransmitter systems. These changes are somewhat similar to those observed in conventional DBS, suggesting that magnetoelectric DBS might be a suitable alternative.

1. Introduction

Deep brain stimulation (DBS) requires invasive stereotactic surgery for the implantation of the electrodes and a tethered pulse generator [1]. Despite its great success in symptom management in movement disorders such as Parkinson's disease (PD) [2–8], DBS has potential surgical

complications such as cerebral hemorrhage and infections [8]. In addition, 15–34% of the patients undergoing DBS procedures require a follow-up surgery for DBS electrode replacement or removal due to hardware malfunctions, displacement, bleeding, or infection [7–9]. For instance, a recent study demonstrated that over 10% of 132 treated patients showed 17 electrode lead migration of more than 3 mm in 16

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patients, due to their dystonic phenotype and problems with the lead fixation at the burr-hole [10]. A minimally invasive DBS system could address some of these challenges and accommodate the growing demand for neuromodulation treatments [11,12]. Among others, several noninvasive neurostimulation techniques have been investigated and used, such as transcranial magnetic stimulation, or transcranial alternating current stimulation for neurological and psychological diseases [13,14]. However, these techniques lack precise targeting and appropriate penetration depth of subcortical structures, such as the subthalamic nucleus (STN) [15]. Recently, we have shown that we can stimulate deep brain targets of mice with wireless nanoelectrodes in vivo [16]. These two-phase magnetoelectric nanoparticles (MENPs) are composed of magnetostrictive and piezoelectric materials, which when strain coupled, generate electric fields in an applied magnetic field. The generated electric field can then elicit specific and local modulation at the injection site [16]. On the other hand, magnetostrictive-only nanoparticles (MSNPs) do not generate electrical fields under a magnetic field and as such, were used as a control [16].

Given these early but promising results, we sought to examine how this novel wireless approach alters the basal ganglia and related circuitry that underlay DBS-related motor and non-motor responses. Clarifying whether this approach induces similar changes in the brain could help establish magnetoelectric DBS as a suitable alternative to conventional DBS. In PD research, subthalamic nucleus (STN)-DBS has been shown to alter the activity of dopaminergic, serotonergic, and cholinergic systems in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), dorsal raphe nucleus (DRN), and pedunclopontine nucleus (PPN) both in healthy and PD conditions [17–20]; reviewed in [21].

The effect of STN-DBS on neuronal activities of dopaminergic SNc neurons has been investigated in several electrophysiological studies in naïve animals [22,23]. Experimental data has shown that STN-DBS decreases the spiking activity in less than half (43%) of the SNc dopaminergic neurons in naïve rats, while increases the spiking activity in another 43% of the dopaminergic cells [22]. However, the effect of STN-DBS was more consistent in PD animals with decreasing spiking activities in 88% of SNc neurons [22]. Another experiment shows that STN-DBS increases the firing rate of 76% of SNc dopaminergic neurons in naïve animals [23]. To date, there is no clear evidence of the effect of STN-DBS on the activity of the VTA dopaminergic neurons in naïve animals. However, the activity of these neurons has been known to be inhibited in response to movement learning behavior activities [24]. In other words, as animals learn to predict rewards, reward-related activity in dopaminergic neurons is decreased [24,25]. Although a few studies have linked the activity of dopaminergic neurons to a particular behavior [26,27], the activity of dopaminergic neurons was somewhat related to the speed of the animal [24].

Previous studies have indicated that STN-DBS inhibits serotonergic neuron activity in the DRN in PD and naïve animals [19,28,29]. Ample evidence suggests that the disruption of the serotonergic raphe system plays a key role in mood disorders [30]. As aforementioned, changes in the activity of the serotonergic system are critical, as it plays an important role in not only the therapeutic but also the adverse effects of DBS. Additionally, both dopaminergic and cholinergic systems are linked to axial symptoms of neurological diseases such as PD [31,32]. Although STN-DBS does not seem to improve all of these axial symptoms [33,34], it is still important to assess whether magnetoelectric DBS could similarly influence the cholinergic system.

Herein, we aimed to address how and to what extent the dopaminergic, serotonergic, and cholinergic systems are altered after magnetoelectric DBS with MENPs in naïve mice. Moreover, we also wanted to assess whether these changes are similar to conventional DBS, and to relate these changes to the behavioral effects observed. Magnetic stimulation was applied to animals injected with either MENPs or MSNPs in the STN. C-Fos co-expression with tyrosine hydroxylase (TH), tryptophan hydroxylase-2 (TPH2), and choline acetyltransferase (ChAT) was

determined with immunohistochemistry (IHC) to assess the activity of dopaminergic, serotonergic, and cholinergic neurons, respectively.

2. Materials and methods

2.1. Animals

Experiments were performed on 16 male naïve mice (C57BL/6 J; the Jackson Laboratory). Animals were housed under constant temperature and humidity with a 12-hour/12-hour dark/light cycle with food access ad libitum. All animal experiments were carried out under a protocol approved by the Institutional Animal Care Committee of Maastricht University in accordance with the Central Authority for Scientific Procedures on Animals.

2.2. Stereotactic nanoparticle injection

The mice were injected with an analgesic (buprenorphine, 0.1 mg/kg s.c.), 30 min prior to the stereotactic surgery. After injection, inhalation anesthesia (isoflurane, Abbot Laboratories, Maidenhead) was induced at 4% and maintained at 1.5–2%. After adequate anesthetic induction, the mouse was positioned in a small animal stereotaxic frame (Kopf, Los Angeles, USA). Body temperature was maintained at 37 °C using a thermo-regulator pad. An ocular ointment was applied to avoid eye dryness. Lidocaine 1% was subcutaneously administered at the incision site as local anesthesia after disinfection of the skin. Burr holes were made into the skull to aim for bilateral STN (AP –2.0 mm, ml ± 1.5 mm, DV –4.5 mm) to inject a total of 2 µl (100 mg/ml) with infusion rate (100 nL/min) of either MENPs or MSNPs using a micro-infusion pump (Nanoject II, Drummond Scientific).

2.3. Magnetic stimulation and behavioral testing

After a 1-week recovery from stereotactic surgery, mice underwent three minutes of magnetic stimulation by applying a 220 mT DC magnetic field with a 6 mT, 140 Hz AC magnetic field to the MENPs and control MSNPs as seen in [16] prior to each behavioral testing. Behavioral tests were performed in a repeated-measures design where both MSNPs and MENPs mice were stimulated in the first trial and then reassigned to off-stimulation in the second trial with a 3-week interval in between the sessions (Fig. 1). Animals were tested in the Catwalk, Rotarod, and Open Field test (OFT). Catwalk and Rotarod testing and data are described and published in our earlier report [16]. For this study, we conducted a follow-up analysis of OFT data and post-mortem immunohistochemistry investigations on animals who underwent behavioral testing in our previous study [16]. Half of the mice in each group were randomly subjected to magnetoelectric DBS (Stim-ON groups) 90 min prior to the perfusion and sacrificing of the animals. The other half served as a control by being placed in the coil while the coil remained off (Stim-OFF groups). The animals were thus sorted into the following groups: MENPs Stim-ON, MENPs Stim-OFF, MSNPs Stim-ON, and MSNPs Stim-OFF, with four mice per group. The experimenter and data analyst were blinded to animal identity during behavioral testing, postmortem histology, and data analysis.

2.4. Open field test

The open field test (OFT) consisted of a clear Plexiglas square arena measuring 100 × 100 cm with 40 cm high walls and a dark floor, as described previously [35]. The OFT measures time spent and the distance moved in the arena to provide an indication of the animal's locomotor activity following magnetoelectric DBS. Animals were individually placed in the center of the arena and were allowed to move freely in the arena for 10 min. The behavior of each mouse was recorded on a computer using the Ethovision tracking software (Ethovision, Noldus Information Technology, Wageningen, The Netherlands). This

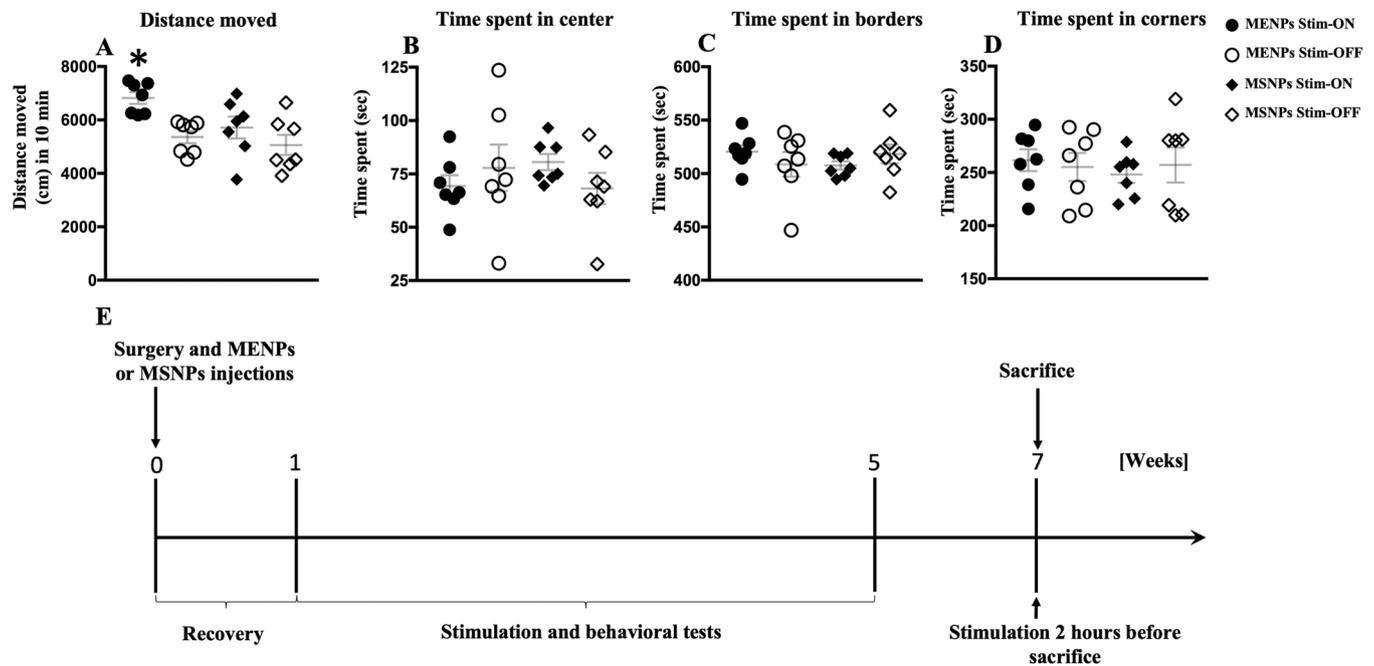


Fig. 1. Magnetic stimulation of MENPs-treated mice in the STN induced behavioral activity changes in the open field test (OFT). A) The graph shows a significant increase in distance moved in mice with magnetic stimulation. B-D) There was no significant difference in time spent in neither the center, borders, nor corners arena in the OFT. E) Timeline of the experiment (Phase-III experiment in our former study [16]). Data are presented as means and \pm SEM; the significant difference ($p < 0.05$) is indicated by an “*”. Abbreviations: Magnetoelectric nanoparticles, MENPs; subthalamic nucleus, STN.

software automatically calculated and analyzed data including the locomotion and distance moved and the time spent in the center, borders, and corners. After each trial, the testing area was cleaned with 70% ethanol solution to diminish the odors of other mice.

2.5. Tissue processing

Mice were deeply anesthetized with pentobarbital and transcardially perfused with Tyrode buffer and 4% paraformaldehyde fixative. Then, brains were extracted and fixed in 4% paraformaldehyde overnight and submerged in 20% sucrose (24 h at 5 °C). The brains were then immediately frozen with CO₂ and stored at -80 °C. After fixation, coronal brain sections (20 μ m) were cut on a cryostat and stored at -80 °C.

2.6. C-Fos immunohistochemistry

Tissue sections series were incubated with a primary antibody raised against c-Fos (rabbit polyclonal; 1:1000; Abcam, ab190289), for two nights followed by a donkey anti-rabbit biotin secondary antibody (Jackson ImmunoResearch Laboratories, west grove, USA, 1:400) and avidin-biotin peroxidase complex (ABC kit Vestastatin, Burlingame, CA, USA; 1:800). The staining was visualized with 3,3'-diaminobenzidine (DAB).

2.7. Double immunofluorescence of tyrosine hydroxylase, tryptophan hydroxylase-2 and choline acetyltransferase with c-Fos

Tissue sections containing the VTA, SNC, DRN, and PPN were incubated overnight with either primary antibodies against TPH2 (Goat polyclonal 1:2000, Abcam, ab121013), TH (Sheep polyclonal 1:2000, Sigma-Aldrich, AB1542), or ChAT (Goat polyclonal 1:200; Sigma-Aldrich, AB144P), respectively in combination with primary c-Fos antibody (rabbit polyclonal Abcam; 1:1000). Donkey anti-goat Alexa 488 and anti-rabbit Alexa 594 secondary antibodies (Jackson ImmunoResearch Laboratories, west grove, USA, 1:200) were used, as well as donkey anti-sheep biotin secondary antibody (Jackson ImmunoResearch

Laboratories, West Grove, USA, 1:200) and streptavidin Alexa 488 (Jackson ImmunoResearch Laboratories, West Grove, USA, 1:5000).

2.8. Quantification of immunohistochemically stained sections

For c-Fos staining, photographs of stained tissue sections containing the motor cortex (MC), the paraventricular region of the thalamus (PV-thalamus), and the centromedial region of the thalamus (CM-thalamus) from three rostrocaudal anatomical levels from Bregma (AP: -0.58, -0.94, and -1.22) were taken at 10X magnification. We used Cell P software (Olympus Soft Imaging Solutions, Münster, Germany) from an Olympus DP70 digital camera with a motorized condenser connected to an Olympus AX70 microscope (Olympus, Zoeterwoude, The Netherlands). In the area of interest, the number of c-Fos cells was counted using ImageJ software [version 1.52; National Institutes of Health (NIH), Bethesda, USA]. A cell was considered positive if the intensity of the cell staining was higher than the surrounding background. In each subject, the average value of three sections was used for statistical analysis.

The double-labeled sections (TH/c-Fos co-expressed in the VTA and the SNC; TPH2/c-Fos in the DRN; and ChAT/c-Fos in the PPN) were analyzed using a fluorescence spinning disk confocal microscope (DSU; Olympus BX51, Hamamatsu City, Japan). 3D virtual tissues were acquired using a digital ultra-high sensitivity CCD camera (C9100-02, Hamamatsu Photonics, Hamamatsu City, Japan). Cell counting was performed in all counting frames using the optical fractionator. Total cell numbers were estimated using a validated stereological method which is previously described [36], and practiced routinely at our laboratory [37].

2.9. Data analysis

Statistical analysis was performed using GraphPad Prism 9.4.0 (GraphPad Software, San Diego, California, USA). Behavior tests were performed in a repeated-measures design where both MSNPs and MENPs mice were stimulated in the first trial and then reassigned to off-

stimulation in the second trial. We performed repeated-measures ANOVA and Bonferroni post-hoc analysis to compare two sets of measurements. Furthermore, immunohistochemical data were analyzed using two-way ANOVA and Bonferroni post-hoc analysis. Data were presented as the mean and standard error of means (\pm SEM), and statistical significance was defined as P -value < 0.05 .

3. Results

3.1. Open field test

In the OFT, MENPs mice showed a significant increase in the distance moved after magnetic stimulation (MENPs Stim-ON: 6822 ± 221 versus MENPs Stim-OFF: 5359 ± 231 , MSNPs Stim-ON: 5715 ± 404 and MSNPs Stim-OFF: 5063 ± 376 cm per 10 min, respectively) compared to the nonstimulated trial and the MSNPs mice [$F(1,12) = 11.78$, $p < 0.05$, pairwise comparison p 's < 0.05 ; Fig. 1A]. However, there was no significant difference in the time spent in neither the center, borders, nor corners in the OFT of all groups [$F(1,12) = 0.10$, $p > 0.05$, $F(1,12) = 0.30$, $p > 0.05$, and $F(1,12) = 0.17$, $p > 0.05$, respectively; Fig. 1B-D].

3.2. Immunohistochemistry

In stimulated mice treated with MENPs, c-Fos expression was significantly increased in the MC (MENPs Stim-ON: 944 ± 65 versus MENPs Stim-OFF: 645 ± 34 , MSNPs Stim-ON: 740 ± 22 and MSNPs Stim-OFF: 737 ± 42 cell count/mm², respectively) and PV-thalamus (MENPs Stim-ON: 678 ± 49 versus MENPs Stim-OFF: 384 ± 45 , MSNPs Stim-ON: 396 ± 12 and MSNPs Stim-OFF: 386 ± 6 cell count/mm², respectively) compared to nonstimulated as well as MSNP-treated

mice [$F(1,12) = 12.04$, $p < 0.01$; Fig. 2A, D-E and $F(1,12) = 20.21$, $p < 0.001$, pairwise comparison p 's < 0.05 ; Fig. 2B, D-E, respectively]. In the CM-thalamus, there was no statistical difference between the groups [$F(1,12) = 0.52$, $p = 0.48$; Fig. 2C-E].

In addition, c-Fos co-expression with dopaminergic, serotonergic, and cholinergic cells was examined using stereological quantification of double-labeled cells. Stimulated mice treated with MENPs showed a significantly lower amount of double-labeled TH/c-Fos cells in the VTA (MENPs Stim-ON: 47 ± 21 versus MENPs Stim-OFF: 276 ± 70 , MSNPs Stim-ON: 309 ± 81 and MSNPs Stim-OFF: 281 ± 28 cells, respectively), compared to nonstimulated as well as MSNP-treated mice [$F(1,12) = 5.82$, $p < 0.05$, pairwise comparison p 's < 0.05 ; Fig. 3A, E-F]. However, no statistical difference was found between groups when analyzing the number of TH/c-Fos cells in the SNc [$F(1,12) = 0.0003$, $p = 0.98$; Fig. 3B, G-H]. In addition, the VTA and SNc TH cell count showed no statistically significant difference between the groups (Fig. 4A-B).

In stimulated mice treated with MENPs, double-labeled TPH2/c-Fos cells in the DRN showed to be significantly decreased (MENPs Stim-ON: 132 ± 33 versus MENPs Stim-OFF: 417 ± 65 , MSNPs Stim-ON: 545 ± 31 , and MSNPs Stim-OFF: 497 ± 77 cells, respectively), compared to nonstimulated as well as MSNPs-treated mice [$F(1,12) = 19.28$, $p < 0.001$, pairwise comparison p 's < 0.01 ; Fig. 3C, I-J]. Quantification of TPH2 cells in the DRN showed no statistically significant difference between groups (Fig. 4C).

Lastly, stimulated mice treated with MENPs revealed no statistical significance in ChAT/c-Fos cells in the PPN between the treatment groups [$F(1,12) = 0.31$, $p = 0.59$; Fig. 3D, K-L]. Quantification of the PPN ChAT-positive cell count showed no statistically significant difference between groups (Fig. 4D). Finally, magnetic stimulation of MENPs-treated mice significantly decreased the c-Fos expression in the VTA [F

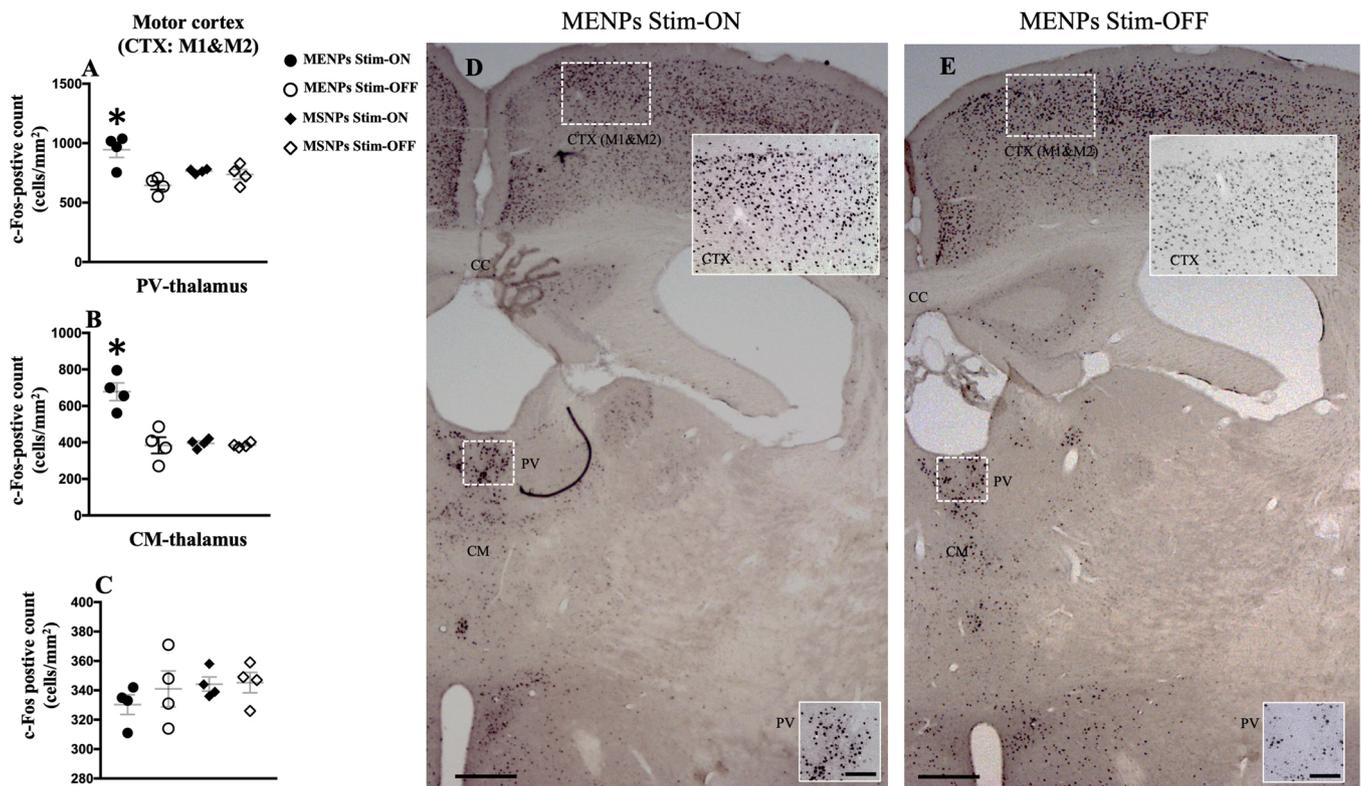


Fig. 2. Magnetic stimulation of MENPs-treated mice in the STN resulted in neuronal activity changes. A-C) Graphs show that magnetic stimulation significantly increased c-Fos expression in the MC and PV-thalamus of MENPs-stim mice, but not in the CM-thalamus. D-E) Representative photomicrographs of coronal sections stained for c-Fos showing the MC, PV- and CM-thalamus, for both stimulated and nonstimulated MENPs-treated mice; scale bar = 250 μ m (overview) and 50 μ m (inset). Data are presented as means and \pm SEM; the significant difference ($p < 0.05$) is indicated by an “*”. Stimulated (Coil-ON), Stim-ON; non-stimulated (Coil-OFF), Stim-OFF; magnetoelectric nanoparticles, MENPs; motor cortex, MC; paraventricular region of the thalamus, PV-thalamus; centromedial region of the thalamus, CM-thalamus.

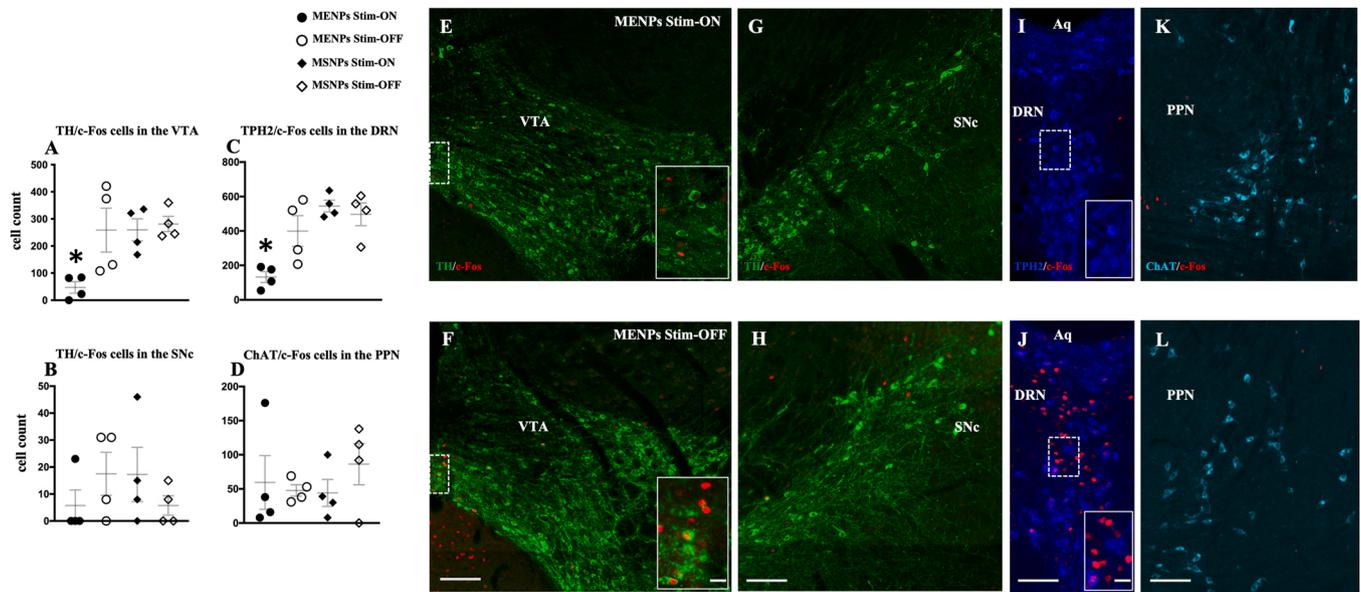


Fig. 3. Magnetic stimulation of MENPs-treated mice in the STN modulates the neuronal activity of the VTA dopaminergic and DRN serotonergic neurons, but neither in the SNc dopaminergic nor the PPN cholinergic neurons. A-B) Graphs show magnetolectric stimulation significantly decreased the TH/c-Fos double-labeled cells in the VTA of MENPs-stim mice, but not in the SNc. It also decreased the TPH2/c-Fos double-labeled cells in the DRN (C) but did not significantly alter the number of c-Fos/ChAT double-labeled cells in the PPN (D). E-H) Representative photomicrographs of coronal brain sections, double-labeled for TH (green)/c-Fos (red) in the VTA and SNc scale bar= 150 μ m (overview) and 15 μ m (inset); I-K) TPH2 (blue)/c-Fos in the DRN; K-L) ChAT (cyan)/c-Fos in the PPN, for both stimulated and non-stimulated MENPs mice; scale bar= 100 μ m (overview) and 15 μ m (inset). Data are presented as means and \pm SEM; the significant difference ($P < 0.05$) is indicated by an “*”. Stimulated (Coil-ON), Stim-ON; non-stimulated (Coil-OFF), Stim-OFF; Ventral tegmental area, VTA; substantia nigra pars compacta, SNc; dorsal raphe nucleus, DRN; pedunculopontine nucleus, PPN; cerebral aqueduct, Aq; tyrosine hydroxylase, TH; tryptophan hydroxylase 2, TPH2; choline acetyltransferase, ChAT. I, J, K, and L) pseudocolours were used for both TPH2 (blue pseudocolor) and ChAT (cyan pseudocolor).

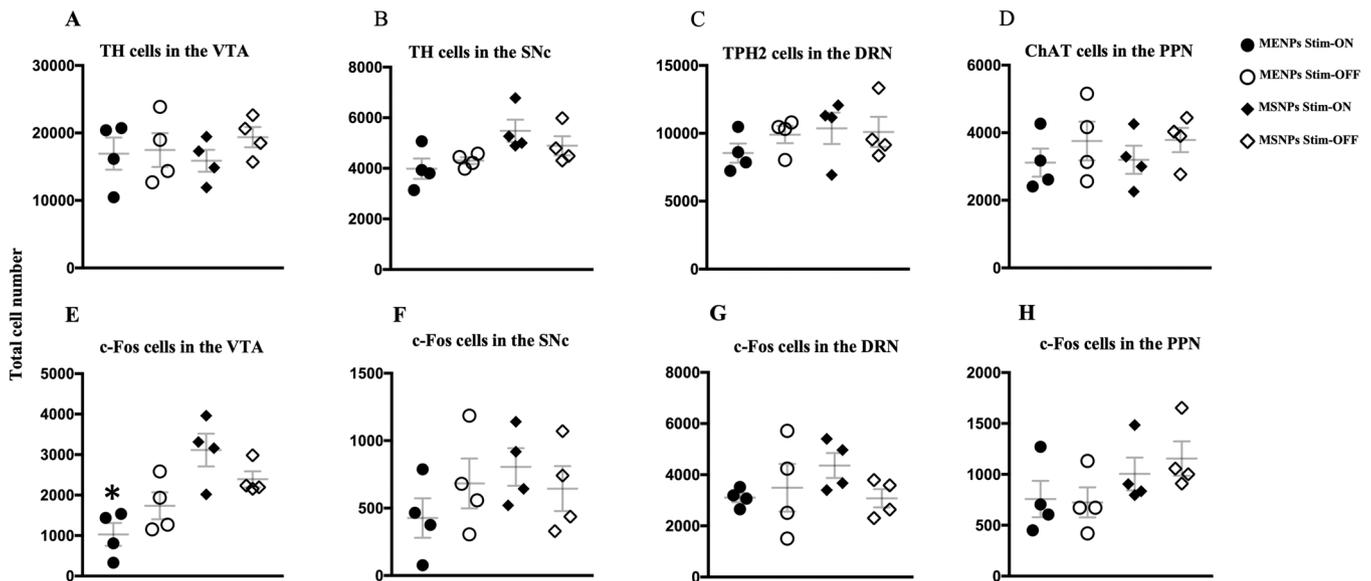


Fig. 4. A-D) Graphs show that magnetic stimulation of MENPs-treated mice in the STN did not significantly alter either the TH, TPH2 or ChAT cell counts in the VTA, SNc, DRN or PPN [$F(1,12)= 0.04$, $p = 0.84$; $F(1,12)= 0.14$, $p = 0.70$; $F(1,12)= 0.35$, $p = 0.57$; and $F(1,12)= 1.87$, $p = 0.20$, respectively]. E-H) Graphs show that magnetic stimulation of MENPs-treated mice significantly decreased the c-Fos expression in the VTA, but not in the SNc, DRN nor PPN. Data are presented as means and \pm SEM; the significant difference ($p < 0.05$) is indicated by an “**”.

(1,12)= 23.18, $p < 0.001$), but not in the SNc, DRN or PPN [$F(1,12)= 0.09$, $p = 0.77$; $F(1,12)= 0.62$, $p = 0.44$ and $F(1,12)= 0.128$, $p = 0.727$, respectively (Fig. 4 E-H)].

4. Discussion

The main goal of this study was to assess the effects of

magnetolectric DBS of the STN on the primary neurotransmitter systems implicated in the working mechanisms of conventional DBS. This is critical for the characterization and validation of this potentially novel DBS approach. Stimulated animals exhibited an increase in c-Fos expression in the MC and PV-thalamus and distance moved in the OFT. Furthermore, TH and TPH2/c-Fos co-expressing cells were reduced in the VTA and DRN, respectively. However, magnetolectric stimulation

did not show a significant difference in the co-expression of ChAT/c-Fos cells in the PPN. Those histological and behavioral findings are somewhat similar to the known effects of high frequency STN-DBS [21].

In this study, we replicated our earlier findings where magneto-electric stimulation increased c-Fos expression in both the MC and the PV-thalamus (Fig. 2A-B, D-E). This explains the behavioral outcomes of our former study, in which enhanced dynamic and speed-related gait parameters were observed in the Catwalk test [16]. Additionally, stimulated animals showed a significant increase in distance moved in the OFT (Fig. 1A). This hyperlocomotion could be due to the effect of magneto-electric stimulation on the activity of dopaminergic cells in the VTA [38–40].

In stimulated animals, we observed a significant reduction in TH/c-Fos double-labeled cells in the VTA, but not in the SNc (Fig. 3A-B, E-H). This indicates that magneto-electric stimulation affects the mesolimbic dopaminergic pathway in naïve animals, while the nigrostriatal pathway is relatively spared. Notably, the TH-expressing cell populations were unchanged in both the VTA and the SNc (Fig. 4A-B). The VTA dopaminergic neurons play an important role in the mesolimbic circuitry, which is implicated in reward, limbic, cognitive as well as psychomotor behavior [41–43].

The STN and its glutamatergic neurons can activate the VTA in the mesolimbic circuitry by neurons in the medial tip of the STN that project to the limbic-related VTA cells [40,44]. Ablation of the VTA with radio-frequency has been shown to induce hyperactivity in non-goal specific movements in rats [38], which is in line with lower dopaminergic neuronal activity and hyperlocomotion observed in this study. In addition, antidromic propagation in the VTA projections, and/or orthodromic activation of GABAergic cells in the VTA or passing-by fibers from the subthalamic area to the VTA could inhibit VTA dopaminergic cells [39,40]. Current literature present ample evidence that challenged VTA dopaminergic system could affect psychomotor behavior [38,43].

We found a selective increase in c-Fos expression in the limbic thalamus (PV-thalamus) and the MC (Fig. 2A-B, D-E). Increased c-Fos expression has also been observed in the MC of naïve rats following electrical STN-DBS [45]. The implications of this regional c-Fos activity pattern on locomotion have been extensively discussed in our previous work [16]. We postulated that the enhanced activity in the PV produces states of arousal that result in hyperlocomotion [46], as it relays information projected from the brainstem and subthalamic areas to the nucleus accumbens and the amygdala, as well as the cortical areas associated with these subcortical regions.

STN-DBS has been shown to elicit debilitating mood effects such as depression, suicide ideation, and impulsivity in some PD patients [47, 48]. Our earlier studies have shown that acute bilateral STN-DBS reduced the firing rate of the DRN serotonergic neurons, decreased serotonin release in the forebrain, and induced depressive-like behavior in PD rats. Given the absence of direct projections from the STN to the DRN, those effects were thought to be relayed via areas such as the lateral habenula [19,49,50]. Moreover, in a recent study, we demonstrated that STN-DBS induces a sustained suppression in the serotonergic system, which was accompanied by depressive-like behavior both in PD and naïve mice [29,51]. These could explain the adverse mood effects following STN-DBS in PD patients, given the fact that the DRN is the main source of serotonin in the central nervous system and its dysfunction has long been associated with the onset of mood disorders [52]. In line with these results, magneto-electric stimulation of the STN inhibited the activity of the serotonergic neurons in the DRN (Fig. 3C, I-J), indicating that nanoelectrode neurostimulation could be comparable to conventional DBS in terms of local and remote network effects.

We also observed that the activity of the cholinergic neurons in the PPN was not altered between groups (Fig. 3D, K-L), despite that there is a known dopaminergic-cholinergic imbalance in axial symptoms of movement disorders, especially in PD [53,54]. A descending projection from the STN to the brainstem and, in particular, the PPN has been described in mammals [55]. However, there is no indication that high

frequency stimulation of the STN influences PPN cholinergic neurons. On the other hand, an optogenetic study has demonstrated that gait improvement in STN-stimulated animals was related to the modulation of upstream connections between the STN and frontal cortices [56]. Likewise, a recent structural connectivity study has attributed the beneficial motor effects of STN stimulation in PD patients to the modulation of fiber tracts between the STN and motor cortex [57], suggesting that STN-DBS does not activate the PPN cholinergic neurons. Similarly, in our study we found no indication that magnetic stimulation in the STN influenced PPN cholinergic neurons. Therefore, the observed motor effects are more likely due to changes in the mesolimbic dopaminergic system rather than the motor circuitry, such as the PPN.

Nevertheless, the current study has some limitations. First, the study was performed in naïve and not parkinsonian animals. Still, this is a necessary first step to understanding the mechanism and effects of MENPs stimulation on the transmitter systems before moving forward to more complex models. While significant work is required to realize this technology as a minimally invasive DBS replacement (e.g., designing the powering device, using less invasive delivery routes) [12], it is important at this technological development stage to explore its effects on local and remote neural elements. Furthermore, in its current state, the proposed technology compromises some freedom that is essential to tailoring the delivery of neuromodulatory effects to the targeted brain region derived from the multiple contacts of the existing DBS lead technology. Further research could explore whether multiple MENPs can be placed and differentially activated to sculpt the volume of activated brain tissue to maximize efficacy and minimize the side-effects.

Despite that, here we report that MENPs stimulation has similar molecular effects to conventional DBS. Comparing the effects of conventional DBS and MENPs stimulation on monoaminergic systems was challenging, especially in naïve animals, as conventional DBS is usually tested in parkinsonian models. Future research will be required to understand these changes more extensively, particularly in PD models, and eventually compare the clinical outcomes of both conventional and MENPs technology, which is the ultimate goal of investigating this novel technique.

5. Conclusion

We have previously demonstrated that magneto-electric nanoelectrodes enable selective modulation of specific brain areas and related behavior in mice [16]. Herein we aimed to investigate the mechanisms of action of wireless DBS compared to known aspects of the conventional DBS mechanisms. We showed that the stimulation of the STN with this approach suppresses the mesolimbic dopaminergic and brainstem serotonergic pathways. These observations are in line with the changes in cell activity as well as animal behavior measured. These changes are comparable to those that have been observed in conventional DBS, suggesting that magneto-electric DBS alters the neural pathways and corresponding behavioural outcomes in a similar fashion, and thus shows promise as a neuromodulatory therapy.

Conflict of interest

All authors declare that they have no conflict of interest.

Data Availability

Data will be made available on request.

Acknowledgment

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