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Rat Behavioral Changes Due to Implanted Magnetic Particles Activated with Externally-Applied Magnetic Fields*

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***Abstract*— While many techniques are available to manipulate deep brain structures (optogenetics, deep brain stimulation, ultrasound, transcranial magnetic stimulation) to treat psychiatric and neurologic disorders, there are issues that remain which decrease clinical utility. For example, DBS is invasive and only able to directly impact a small portion of the brain at a time. Recent data has shown that mechanical stimulation of neurons depolarizes the cells increasing their firing rate. Here we implanted magnetic nanorods into neural tissue of live rats. We then designed a magnetic coil to provide external magnetic stimulation to the implanted nanorods while rats were awake and ambulatory.**

Male rats were stereotactically implanted with magnetic nanorods. Control animals experienced sham surgeries. One week following surgery, animals were placed in a small open space within a coil. Low-magnitude (5 mT, 20 Hz) external magnetic stimulation was applied. Total locomotion, rotations, and chewing behaviors of the rats for the duration of the experiment (7 minutes) were measured. Animals with implanted nanorods showed significantly increased levels of chewing behavior compared to sham controls. These data demonstrate the ability of mechanical stimulation of striatal neurons to modify rat behavior. Potential future applications of the technology include the use of wearable generators of low magnetic fields applied to intra-nasally administered magnetic nanoparticles.

I. Introduction

Deep brain stimulation (DBS) with implantable electrodes is FDA approved for the treatment of movement disorders such as Parkinson's disease and has been suggested for use in other psychiatric diseases such as depression and addiciton [1, 2]. The use of such deep-brain stimulation procedures has been problematic because of concerns about invasiveness of the procedure and sizes of the probes. We propose methods of modulating behavior using implanted vibrating magnetic nanorods that mechanically stimulate nearby neurons. The nanorod vibration is affected by exposure to oscillating or pulsatile magnetic fields generated by external magnets. Mechanical neuronal stimulation was previously demonstrated by large scale motion of neural probes [3]. Previous *in vitro* work has shown that externally applied magnetic fields can increase neuronal firing rates in cells loaded with magnetic particles *in vitro [4]*. Here, we implant magnetic nanorods into rat striatal tissues and observe rat behavior before, during, and after magnetic activation from external sources. We grade animal behavior for a specific behavior before, during, and after magnetic activation, and compare rats with implanted nanorods against control rats that received a sham surgery.

II. Materials and Methods

A. Synthesis of nanorods

Nanorods are grown via template guided electroplating using anodized aluminum oxide (AAO) membranes as the growth templates (Fig. 1) [5, 6]. AAO templates (13 mm diameter) contain pores which are about 250-nm in diameter and are open on both sides of the template. One side of the template is sealed by thermal evaporation of a 550 nm thick layer of silver. The template is then placed in a growth chamber, sealing the silver surface in contact with a working electrode (cathode). The remaining open pores of the template are exposed to any fluid filling the growth chamber. The growth chamber is then filled with an electrolyte containing a single metallic species. Using a wound platinum wire as the anode, nanorod growth occurs inside the pores of the AAO template via DC electroplating of metallic layers. Au-Fe-Au multilayered nanorods are grown by sequentially changing

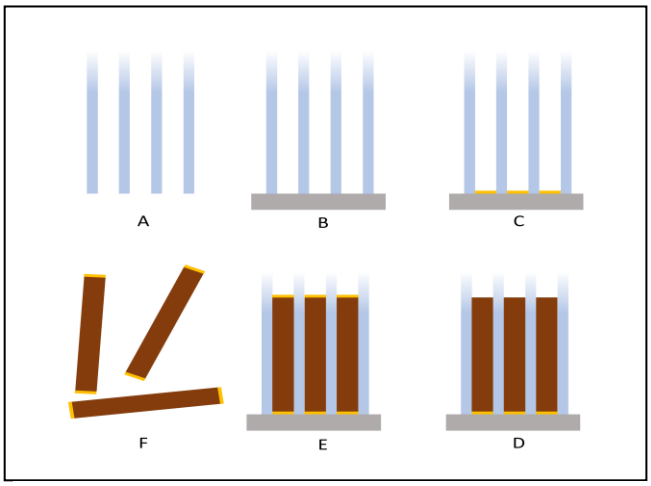

Fig. 1. Particle manufacturing process begins with template (A) that is electroplated (B), successively filled (C-E) and then dissolved to release particles (F). Blue represents template; Gray represents thermally evaporated silver layer; Yellow represents electrodeposited gold layer; brown represents electrodeposited iron layer. Rods are released by first etching the thermally evaporated Ag layer in 30% nitric acid, then etching the AAO in 0.5 M NaOH.

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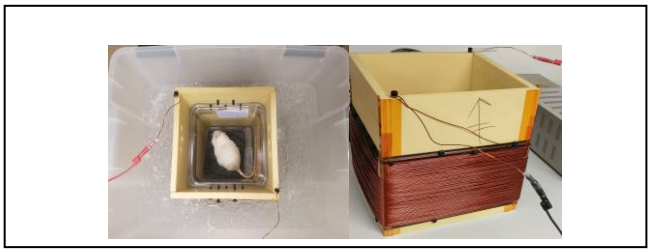

Fig. 2. Behavioral Apparatus inside Magnetic Coil. A picture of a rat inside the small open field where behavior measures were recorded alongside a picture of the outside of the coil. The coil is wrapped using AWG 16 wire and is 30 cm on a side and 15 cm tall.

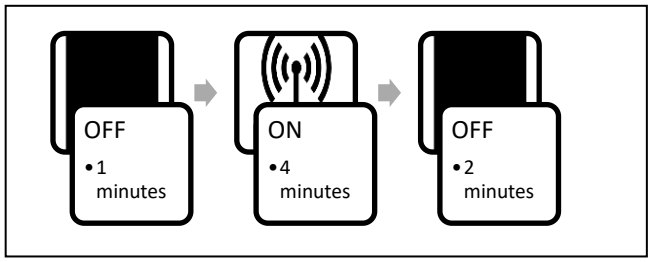

Fig. 3. External Magnetic Stimulation and Animal Behavior Protocol Design. Animals were first placed in the small open field for 1 minute. Magnetic stimulation was applied for 4 minutes. Two additional minutes were recorded following stimulation.

the electrolyte (Fig. 1). Referring to Fig. 1, (A) AAO template (blue) containing pores (B) is first sealed on one side with a thermally evaporated layer of silver (gray). (C) A thin layer of gold is electroplated, (D) followed by a segment of iron, (E) followed by a capping l ayer of gold. (F) The silver layer and AAO are etched, releasing the Au-Fe-Au rods.

Gold was grown using commercial gold plating solution (Orotemp 24 RTU, Technic, Inc.), and iron layers were grown using an electrolyte solution containing 120 g/L $FeSO_4.7H_2O$, 45 g/L H_3BO_3, and 1 g/L ascorbic acid [7]. Iron plating solution was <12 hours old at time of synthesis. Au layers are <50 nm long and Fe layers are 5 µm long. Section length is determined by deposition time and current. Typical growth parameters for our nanorods are ~0.9 mA and ~1.6 VDC. After growth, the silver working electrode was etched using nitric acid and the AAO was etched using 0.2 M NaOH.

Five rinsing and resuspension cycles were performed to clean the Au-Fe-Au nanorod sample. Removal of dissolved AAO is confirmed by optical microscopy, during which no undissolved AAO was observed. Each rinsing cycle removes 99% of the solution, replacing it with fresh DI water. Thus, each rinsing cycle reduces AAO concentration by two orders of magnitude. Nanorods were stored in ethanol prior to use. Prior to injection, nanorods were transferred to phosphate buffered saline (PBS) and rinsed five times with PBS and alternating sonication and centrifugation to ensure all ethanol was removed. Rods were sterilized prior to implantation. Rods are 5 µm in length (+/- 30 nm) and 250 nm wide (+/- 10 nm). Rods were then injected under stereotaxis into the brains of anesthetized rats. Each rat was dosed with one million particles in 1 µl volumes. Control animals received 1 µl phosphate buffered saline.

B. Magnetic Coil Design

A stimulation coil was designed to provide a volume sufficient for an awake, ambulatory rat to move around (Fig. 2). Our magnet was wound around a rectangular box composed of nylon sheet (12 cm thick) using AWG 16 HML magnet wire (MWS Wire). The magnet was 30 cm on a side, 15 cm tall, had 3 layers each containing 100 turns, and had a resistance of 4Ω. A function generator (Siglent Technologies, SDG2042X) supplied a continuous wave sinusoidal signal (20 Vpp, 20 Hz) amplified with an audio amplifier (QSC RMX 4050a) operated in bridge mode.

C. Animal Procedures

Animals: Male Sprague Dawley rats weighing 250-300 g were obtained from Charles River Breeding Laboratories. Animals were housed in groups of 3 with food and water available *ad libitum*. A 12/12 hr light/dark cycle was used with the lights on at 7:00 am. All experimental procedures were performed during the light cycle. All procedures were approved by the Institutional Animal Care and Use Committee of Tufts University and were carried out in accordance with the National Research Council (NRC) Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal distress.

Stereotaxic particle injections: Animals were anesthetized with 80mg/kg ketamine and 4 mg/kg dexmedetomidine (i.p.). Meloxicam was administered as a presurgical analgesic (1 mg/kg, s.c.). Rats were mounted in a stereotaxic apparatus (Kopf Instruments, CA) and a unilateral hole drilled in the skull according to coordinates determined from Paxinos and Watson rat brain atlas (A/P +1.5mm, M/L +2.5mm). Small plastic microinjectors attached via plastic tubing to a Hamilton syringe were lowered into the striatum (D/V -5.0mm). Dissolved particles (1 million/µl) or vehicle (phosphate buffered saline) were slowly injected over 2 minutes. Microinjectors were left in place for an additional minute to allow for diffusion away from the tip of the injector. Animals were allowed to recover for 1 week following injections.

Behavioral Analysis: Each animal was placed in the small open field for 1-minute for acclimation (PRE) (see Figure 3). External magnetic stimulation was then applied for 4 minutes. The animal remained in the field for 2 additional minutes following stimulation. All behaviors were recorded with a digital camera for the entire 7-minute task. Videos were scored by a blinded observer. Total locomotor activity, rotations, and chewing behavior were scored. All experiments were repeated twice. In the first trial (Trial 1), there were 7 animals with nanorods and 5 shams. In the second trial, there were 8 animals per group.

III. Results

There were no differences in total distance traveled between the two groups or in total number of rotations before, during, or after magnetic stimulation (data not shown). There were differences in the amount of time the animals with implanted nanorods spent chewing compared to sham control s. Fig. 4 shows the ratio of chewing compared to not chewing during the on and off stimulation phases, characterized by mean +/- standard error. The on period represents the second two minutes of stimulation on. The off period represents the two minutes following stimulation.

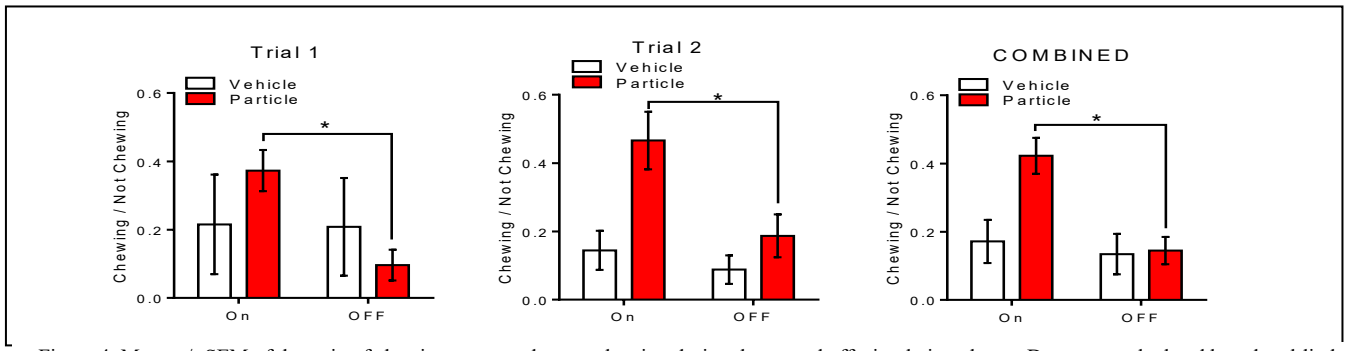

Figure 4. Mean +/- SEM of the ratio of chewing compared to not chewing during the on and off stimulation phases. Data were calculated based on blinded video recordings. The on period represents the second two minutes of stimulation on. The off period represents the two minutes following stimulation. *p<0.05 Off vs On for particle injected animals

Data were analyzed with a two-way ANOVA, which revealed a main effect of stimulation, main effect of particle, as well as an interaction for the combined data set. Main effect stimulation $F(1,26) = 13.82$, $p<0.001$. Main effect particle $F(1,26)=4.28$, $p<0.05$. The interaction $F(1,26)=8.05$ had a $p<0.01$. Sidak's multiple comparison post hoc analyses revealed that the ratio of chewing during the off phase was significantly less than the ratio of chewing during the on phase ($p<0.0001$). The Off vs On for particle injected animals had a $p<0.05$. These data show that the animals with nanorods spent more time chewing than the sham controls

We analyzed the same chewing data in different ways to try to gain a better understanding of the entire behavior. Fig. 5 shows the Mean +/- SEM Chew score for vehicle and particle treated animals. Chew score was calculated by determining the percentage of chewing during stimulation on periods and the percentage of chewing during stimulation off periods was subtracted from that number. Data were analyzed with a student's t-test, which reached statistical significance (*$p<0.05$).

We also wanted to gain an understanding of the timecourse of the effect to determine if the effect increased across stimulation or showed any residual effects once stimulation ceased. Fig. 6 shows Mean +/- SEM of the percent time chewing across the entire 7-minute behavioral test. Percent time chewing was calculated by dividing the amount of time spent chewing by the total amount of time in that bin. The time was divided into 4 bins: The first OFF period was 1-minute long. The 4-minute ON period was split into 2 two-minute bins. The final OFF period was 2 minutes long. Data from the first and second trial are shown as well as the combined data set. Data were analyzed with a repeated measures two-way ANOVA with time as the repeated measure and particle vs vehicle as the between-subjects factor. Data reveal a significant interaction in trial 1, a

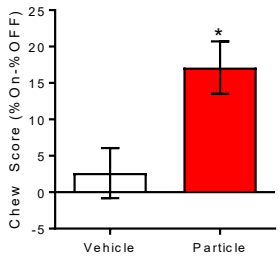

Fig. 5. Mean +/- SEM Chew score for vehicle and particle treated animals. *p<0.05 Particle injected animals compared to vehicle injected animals

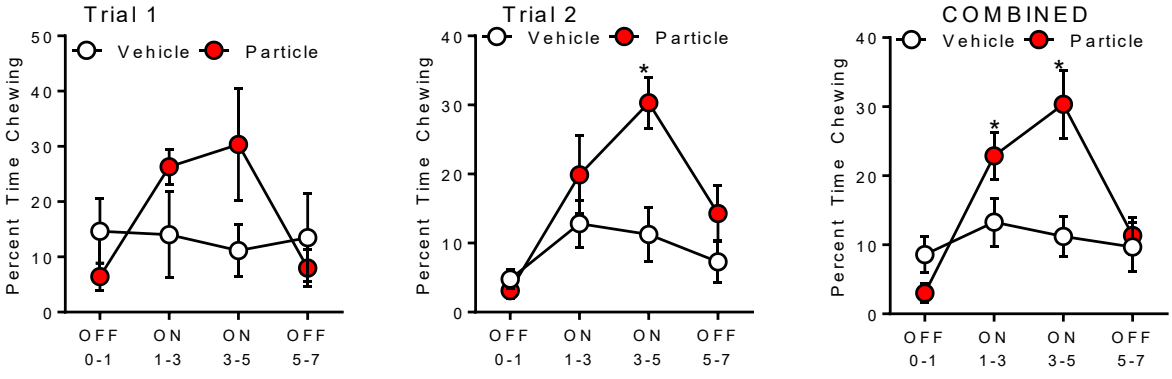

Fig. 6. Time course of experiments. Mean +/- SEM of the percent time chewing across the entire 7-minute behavioral test. Percent time chewing was calculated by dividing the amount of time spent chewing by the total amount of time in that bin. The time was divided into 4 bins: The first OFF period was 1-minute long. The 4-minute ON period was split into 2 two-minute bins. The final OFF period was 2 minutes long. Data from the first and second trial are shown as well as the combined data set. * $p<0.05$ compared to sham controls.

significant effect of particle, stimulation and an interaction in the trial 2 and significant effect of stimulation and an interaction in the combined data set. (combined: Stimulation: $F(3,78)=12.83$; $p<0.0001$; interaction: $F(3,78)=7.64$; $p<0.001$). Sidak's post hoc analyses revealed a significant impact of stimulation during the last 2 minutes of the ON test.

IV. DISCUSSION

Combined, these results show that external magnetic stimulation of particles implanted in the dorsal striatum can icrease the amount of time that an animal engages in chewing behavior. Chewing behavior is a typical exploratory behavior observed in rodents. However, vacuous chewing movements, undirected or excessive chewing behavior is abnormal and represents a significant impact on neuronal activity caused by mechanical stimulation of striatal neurons. The physiological changes in the medium spiny neurons of the striatum are unknown, however, there are known pharmacological mechanisms that cause increased vacuous chewing movements or oral facial dyskinesias and stereotypy. For example, increased vacuous chewing are a hallmark of a D2 antagonist actions in the striatum and associated with side effects common in antipsychotic treatments. Indeed, chronic treatment with typical antipsychotic medications can lead to tardive dyskinesia, thought to result from full D2 receptor occupancy with antagonist properties [8, 9]. This might indicate that mechanical stimulation is causing downstream changes reminiscent of D2 antagonists.

Orofacial dyskinesias are also observed as a common side effect of levodopa therapy in Parkinson's disease. Levodopa is a replacement therapy and increases dopamine stimulation or neuronal activation in the striatum. The mechanism for dyskinetic side effects may involve the dopamine D1 or D3 receptors [10]. In addition, there is a body of literature that suggests that the dopamine system is involved in the control of licking and grooming behaviors, which may become hijacked in the case of stereotypy's and dyskinesias [11]. The location of the particle stimulation was the dorsal medial striatum, which consists of medium spiny neurons and interneurons. The medium spiny neurons contain either D1-like or D2-like receptors and unilateral mechanical stimulation may activate or inhibit specific intracellular signaling pathways that mimic the effects of receptor occupancy with ligand. The studies are consistent with cellular studies demonstrating physiological response of mechanical particle stimulation on neuronal cultures and crayfish [12], as well as other work in which carbon fiber microelectrodes were used to induce adenosine release via mechanical stimulation [13]. Thus, it is clear that mechanical stimulation of striatal neurons is having an impact on neuronal physiology and future experiments are necessary to determine the precise mechanism of action. Similar magnetic particles have been transported intra-nasally into the brain and concentrated deep in selected locations using pulsed magnetic fields, suggesting that the vision of a system for non-invasive administration and image-guided delivery to specific brain foci is becoming closer to reality [13, 14]. Combination with a programmable and wearable component for delivering magnetic fields would improve access as compared to competing technologies (e.g. high intensity focused ultrasound).

Additional work (e.g. safety studies) will be required to translate the technique into the clinic. Studies of prior rods have suggested that frustrated phagocytosis can occur if rods are larger than 12 μm in length (which is larger than the rods in this study) [14]. The particles can be built to dissolve after several weeks [12], which would be expected to reduce toxicity. Our vision is that patients with particles localized to specific brain foci (e.g. nucleus accumbens, implicated in addiction) could receive programmed stimulation with wearable magnetic field generators as an alternative to drugs.

V. CONCLUSION

The finding that mechanical stimulation of striatal neurons can change behavior in rodents leads to the exciting possibility that wearable magnetic field generators in combination with magnetic nanoparticles could be utilized in the treatment of neurologic and psychiatric diseases.

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