Wireless magnetothermal deep brain stimulation

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Wireless deep brain stimulation of well-defined neuronal populations could facilitate the study of intact brain circuits and the treatment of neurological disorders. Here, we demonstrate minimally invasive and remote neural excitation through the activation of the heat-sensitive capsaicin receptor TRPV1 by magnetic nanoparticles. When exposed to alternating magnetic fields, the nanoparticles dissipate heat generated by hysteresis, triggering widespread and reversible firing of TRPV1+ neurons. Wireless magnetothermal stimulation in the ventral tegmental area of mice evoked excitation in subpopulations of TRPV1+ neurons, demonstrating minimally invasive and remote neural excitation through the activation of TRPV1+ neurons. These findings suggest that magnetothermal stimulation could facilitate the study of intact brain circuits and the treatment of neurological disorders.
relevant alternating magnetic field conditions can (i) reduce the latency period for neural excitation, (ii) eliminate exogenous targeting transgenes, and (iii) have chronic utility in vivo because MNPs exhibit minimal cytotoxicity and remain intact several months after injection (12, 13). Spherical Fe3O4 MNPs 22 nm in diameter possess some of the highest heating rates per gram, or specific loss power, measured for a synthetic material at a therapeutically relevant frequency \( f = 500 \text{kHz} \) and field amplitude \( H_o = 15 \text{kA/m} \) (14). We prepared these monodisperse MNPs via the iron-oleate precursor (15) and dispersed them in water through high-temperature ligand exchange with poly(acrylic acid) (PAA) (Fig. 1B) (14). Grafting poly(ethylene glycol) (PEG) chains onto PAA-coated MNPs resulted in their steric dispersion, which improved colloidal stability (Fig. 1, C and D) and biocompatibility, as indicated by the increased viability of human embryonic kidney (HEK) 293FT cells over prolonged exposure (Fig. S1) (16). These MNPs exhibited specific loss of power of 660 ± 50 W/g, which is sixfold greater than that of hyperthermia agents currently used in clinical settings (Fig. S2). Magnetic fields were generated by a resonant coil custom designed for fluorescence imaging during stimulation (fig. S3, A to E). Although transient receptor potential cation channel subfamily V member 1 (TRPV1) is naturally expressed across the mammalian nervous system (17), we designed a transgene to establish sustained and uniform levels of TRPV1 expression for magnetothermal membrane depolarization across different cell lines (18). The TRPV1 transgene was placed under the excitatory neuronal promoter calmodulin kinase II \( \alpha \)-subunit along with mCherry separated from TRPV1 by the posttranscriptional cleavage linker p2A (CamKII\(\alpha\):

\text{TRPV1-p2A-mCherry}) (19) and packed into the lentiviral vector so as to enable long-term expression for magnetothermal membrane depolarization (20). Cells were additionally transfected with the adeno-associated virus serotype 9 (AAV9) carrying GCaMP6s under the neuronal promoter human synapsin (hsyn::GCaMP6s) for measurement of intracellular Ca2+ changes as a proxy for membrane depolarization (21). Functionality of the two genes was confirmed by observing increased fluorescence intensity in response to capsaicin, a TRPV1 agonist, and temperature increase above 45°C in nonexcitable HEK293FT cells (fig. S4, A to C).

We first demonstrated magnetothermal control of intracellular Ca2+ influx in HEK293FT cells. Fluorescence intensity maps indicated that only cells expressing TRPV1 (TRPV1+) responded to the field stimulus (\( f = 500 \text{kHz} \), \( H_o = 15 \text{kA/m} \)) when incubated in MNP solutions (2 mg/ml), whereas cells not expressing TRPV1 (TRPV1-) as well as TRPV1+ and TRPV1- cells without field stimulus did not exhibit changes in intracellular Ca2+ concentration (Fig. 1E). A field-induced temperature increase in excess of 43°C in MNP solutions triggered a GCaMP6s fluorescence increase of \( \Delta F/F_0 > 50\% \) in 36.1 ± 4.3% (mean ± SD) of TRPV1+ cells, whereas only 1.7 ± 1.6% (mean ± SD) of TRPV1- cells exhibited a similar response (Fig. 1F and fig. S5, A to D).

Magnetothermal membrane depolarization was sufficient to evoke trains of action potentials in primary hippocampal neurons expressing TRPV1 when exposed to 10-s field pulses at 60-s intervals. Viral transfection with AAV9-hsyn::GCaMP6s, which allows for fluorescence detection of single action potential events (21), and Lenti-CamKII\(\alpha\):

\text{TRPV1-p2A-mCherry} (TRPV1+) or Lenti-CamKII\(\alpha\):

\text{mCherry} (TRPV1-) yielded a coexpression efficiency of 57% after 5 days (Fig. 2A). In MNP solutions (10 mg/ml), 85 ± 14% of TRPV1+ neurons exhibited synchronized firing within 5 s after stimulus, whereas only sporadic activity was observed in TRPV1- neurons (TRPV1-) when exposed to the field stimulus (\( f = 500 \text{kHz} \)).

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Fig. 2. Alternating magnetic field stimulus evokes correlated and repeated trains of action potentials. (A) Confocal fluorescent images of cotransfected hippocampal neurons. Scale bar, 25 \( \mu \text{m} \). (B) Population study of 100 neurons from three trials counting the number of neurons that spike within a 5-s bin after magnetic field stimulus. (C and D) Temperature profiles during magnetic field application in Tyrode’s solution (C) without and (D) with MNPs. Shaded area is the SD with average value overlaid (black). (E to H) Example fluorescence traces of 10 individual neurons with average overlaid (black). (I to L) Raster plots of 100 randomly selected neurons from three trials. Calcium spikes were counted according to an automated algorithm. (M to P) Peristimulus time histograms of the raster plots binned at 2 s. Color scheme for (E) to (P); TRPV1+ neurons in Tyrode’s solution without MNPs, gray; TRPV1+ neurons in Tyrode’s solution with MNPs, red; TRPV1- neurons in Tyrode’s solution without MNPs, blue; TRPV1- neurons in Tyrode’s with MNPs, orange. Shaded blue bars represent alternating magnetic field pulses (\( H_o = 15 \text{kA/m}, f = 500 \text{kHz} \)).
was observed in TRPV1+ neurons (Fig. 2, B to H). This implies that the temperature increase (Fig. 2D) in MNP solutions exposed to alternating magnetic field was sufficient to trigger TRPV1 (Fig. 2H) while avoiding nonspecific thermal effects such as changes in membrane capacitance (Fig. 2F) (22). In the absence of MNPs, magnetic field did not induce appreciable solution heating (Fig. 2C), and no correlated response was observed in TRPV1+ or TRPV1- neurons (Fig. 2, B, E, and G). We recorded neural activity from GCaMP6s temporal fluorescence traces (Fig. S8, A to D, and movie S1) (23). Waves of Ca2+ spikes were repeatedly induced by field pulses only in TRPV1+ neurons in the presence of MNPs (Fig. 2, I to P). The observed 5-s latency between the field application and the onset of neural activity is fivefold faster than previously described (8).

We next tested whether alternating magnetic field could activate a subpopulation of neurons in deep brain tissue in mice. We used finite element modeling corroborated with temperature recordings in brain phantoms to predict local temperature changes in response to field stimulus (Fig. S7). Injections (2.5 μL) of MNP solution (100 mg/mL) delivered temperature gradients sufficient to reach the TRPV1 activation threshold within 5 s and cool back to 37°C over 60-s cycles (Fig. S7, B to F), thus avoiding prolonged exposure to noxious heat (Fig. S7G) (24).

With low endogenous expression of TRPV1 (25) and well-characterized projections (26), the ventral tegmental area (VTA) was an attractive deep brain target for initial demonstration of magnetothermal stimulation. Furthermore, phasic excitation in the VTA has therapeutic implications in the treatment of major depression (27). We sensitized excitatory neurons in the VTA to heat through the lentiviral delivery of TRPV1, which was followed by MNP injection into the same region 4 weeks later (Fig. 3, A and B, and fig. S8A). The anesthetized mice were exposed to the magnetic field conditions described above (Fig. S8, B and C). Neuronal excitation was quantified by the extent of activity-dependent expression of the immediate early gene c-fos within a 250-μm vicinity of the MNP injection (Fig. 3, C to F) (28). Neural activity was only triggered by magnetic field in the VTA of mice transfected with TRPV1 in the presence of MNPs, resulting in a significantly higher proportion of c-fos-positive (c-fos+) cells, as revealed by a two-way analysis of variance (ANOVA) with a Bonferroni post hoc test (Fig. 3G). Control subjects testing whether the MNP injection, heat dissipation with field stimulus, or TRPV1 expression alone can result in neural stimulation showed no significant c-fos expression (Fig. 3, C to E and G). Furthermore, the spatial extent of neuronal activation was largely collocated with TRPV1 expression in the VTA (Fig. 3, H and I).

We next investigated whether neurons in the VTA can be activated 1 month after MNP injection so as to explore its chronic utility (Fig. 3, J to O). We again observed increased c-fos expression in the VTA only in mice transfected with TRPV1 in the presence of MNPs and exposed to

**Fig. 3. Wireless magnetothermal stimulation in vivo.** (A) In vivo experimental scheme. (B) Confocal image of a coronal slice representative of the TRPV1-p2A-mCherry expression profile in the VTA. (C to F) TRPV1+ VTA 4′,6-diamidino-2-phenylindole (DAPI) (blue), mCherry (red), and c-fos (green) and overlay confocal images of regions used for quantification of neural stimulation. Scale bar, 25 μm. All animals were injected with MNPs. Experimental conditions were (C) without (OFF) and (D) with (ON) magnetic field stimulation in TRPV1+ VTA, and (E) OFF and (F) ON stimulation in TRPV1+ VTA. (G) Percentage of mCherry-positive and c-fos-positive neurons within cell population indicated by DAPI corresponding to the four conditions presented in (C) to (F). Significance is confirmed by two-way ANOVA with Bonferroni post hoc test (n = 4 mice, F1,13 = 47.5, P < 0.0001). (H and I) Confocal images of the VTA after acute magnetothermal stimulation. c-fos expression is largely confined to the VTA in regions where TRPV1 is expressed. Scale bar, 100 μm. (J to L) Confocal images of the (J) VTA, (K) mPFC, and (L) NAc 1 month after MNP injection without (OFF) and with (ON) field treatment. Scale bar, 100 μm. (M) Percentage of c-fos+ neurons in the VTA among DAPI-labeled cells with and without magnetic field stimulation. Increased c-fos expression is observed after field treatment (ON) as compared with unstimulated (OFF) controls (n = 3 mice OFF/ON; Student’s t test, P < 0.02). (N and O) Similarly, up-regulation is observed in the NAc the mPFC and (O) in the NAc with alternating magnetic field (ON) as compared with the same regions without (OFF) the field stimulus (n = 3 mice OFF/ON; Student’s t test *P < 0.02, **P < 0.002).
Intergenerational transmission of child abuse and neglect: Real or detection bias?

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The literature has been contradictory regarding whether parents who were abused as children have a greater tendency to abuse their own children. A prospective 30-year follow-up study interviewed individuals with documented histories of childhood abuse and neglect and matched comparisons and a subset of their children. The study assessed maltreatment based on child protective service (CPS) agency records and reports by parents, nonparents, and offspring. The extent of the intergenerational transmission of abuse and neglect depended in large part on the source of the information used. Individuals with histories of childhood abuse and neglect have higher rates of being reported to CPS for child maltreatment but do not self-report more physical and sexual abuse than matched comparisons. Offspring of parents with histories of childhood abuse and neglect are more likely to report sexual abuse and neglect and that CPS was concerned about them at some point in their lives. The strongest evidence for the intergenerational transmission of maltreatment indicates that offspring are at risk for childhood neglect and sexual abuse, but detection or surveillance bias may account for the greater likelihood of CPS reports.

For years, the notion that abused children grow up to become abusive parents has been widely accepted in the field of child abuse and neglect (J–9). However, because many other factors in a person’s life (such as natural abilities, biological or genetic predispositions, or intervening relationships) may mediate the effects of child abuse and neglect, assessing the intergenerational transmission of abuse and neglect is challenging. Although some studies have provided empirical support for the intergenerational transmission of child abuse (4–10),

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SUPPLEMENTARY MATERIALS
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Materials and Methods
Supplementary Text
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References (34–47)
Video S1
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