

Review

Magnetic nanomaterials for wireless thermal and mechanical neuromodulation

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SUMMARY

Magnetic fields are very attractive for non-invasive neuromodulation because they easily penetrate through the skull and tissue. Cell specific neuromodulation requires the magnetic field energy to be converted by an actuator to a biologically relevant signal. Miniaturized actuators available today range from small, isotropic magnetic nanoparticles to larger, submicron anisotropic magnetic nanomaterials. Depending on the parameters of external magnetic fields and the properties of the nanoactuators, they create either a thermal or a mechanical stimulus. Ferromagnetic nanomaterials generate heat in response to high frequency alternating magnetic fields associated with dissipative losses. Anisotropic nanomaterials with large magnetic moments are capable of exerting forces at stationary or slowly varying magnetic fields. These tools allow exploiting thermo-sensitive or mechanosensitive neurons in circuit or cell specific tetherless neuromodulation schemes. This review will address assortment of available magnetic nanomaterial-based neuromodulation techniques that rely on application of external magnetic fields.

INTRODUCTION

In the past decade, neuroscientists have made significant progress in linking neural activity and brain circuits to behavior and behavioral responses. This breakthrough was facilitated by an expanding array of tools able to activate or inhibit neurons with cell type-specificity on demand (Figure 1 and Table 1). These tools have in common that they use nano- or microstructures to convert physical or chemical energy into a stimulus and genetic approaches to sensitize specific neurons in the brain to the stimulus (Figure 2 and Table 1).

The most established method, optogenetics, relies on microbial rhodopsins to sensitize genetically targeted neurons to visible light. The trigger light is delivered from the outside via implanted glass fibers or by remotely activated miniature LEDs implanted in the brain. Optogenetics can provide cell population specific neuromodulation with high temporal precision. Chemogenetics eliminates the need for surgical implantation by using the binding of a diffusible molecule to designer receptors exclusively activated by designer drugs (DREADD) as stimulus. However, the resulting stimulus has very limited temporal precision (Gomez et al., 2017). Moreover, recent data indicate reverse metabolization of clozapine-N-oxide (CNO) to clozapine and production of interoceptive stimulus effects in rodents (Manvich et al., 2018). Several non-genetic, non-invasive methods such as transcranial magnetic stimulation (TMS) and focused ultrasound (fUS), provide translation potentials, but do not discriminate between cell types. In addition, the resolution of these techniques is inversely proportional to penetration depth, limiting access to modulation of cortical layers (Wassermann, 2000; King et al., 2013). Furthermore, the cellular and molecular biophysical mechanisms causing the neuromodulation are not fully understood, complicating the application and interpretation of the results (Garcia-Lazaro et al., 2015).

A main advantage of using nanomaterials for neuromodulation is in minimally invasive interventions for implantation. Compared to micro/macro scale counterparts such as microelectrodes, they don't require stiff connection between skull and brain tissue. Micro and microscale stiff electrodes lead to glial scarring impacted by brain micromotions, which is avoided when using nanosized materials. Nanomaterials are sufficiently small to diffuse into gaps between cells, reaching their target easily. Hence, they allow for interfacing the nervous system at cellular and molecular levels where conversion of input energy to neural stimulus occurs due to their magnetic, optical or electrical properties (Figure 1 and Table 1). Furthermore, the surface of nanoparticles is easily functionalized to increase biocompatibility and enable specific targeting.

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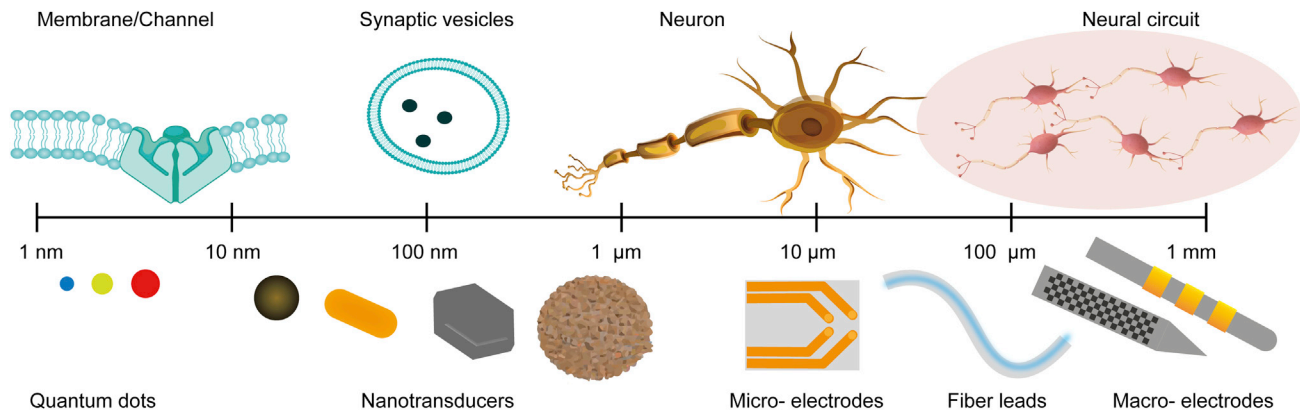


Figure 1. Comparison of neuromodulatory materials with the size of interacting biological entities

Using magnetic fields coupled to magnetic nanoparticles is an attractive approach for remote control of neurons as it provides cellular resolution and the mechanism of activation is clear. Magnetic fields can easily penetrate through skull and tissue where the energy of magnetic field is converted by a nanoscale actuator to a biological stimulus. Huang et al. demonstrated that heat dissipated by magnetic nanoparticles under alternating magnetic fields of <50 mT and hundreds of kHz can trigger temperature-sensitive ion channels and activate cultured neurons (Huang et al., 2010). Chen et al. showed the capability of magnetothermal stimulation to activate deep brain neurons of rodents (Chen et al., 2015). Application of magnetothermal neuromodulation demonstrated ability to control robust behavioral responses in freely moving rodents (Munshi et al., 2017). Both Chen et al. (2015) and Munshi et al. (2017) studies showed stability of non-targeted NPs in the brain for several months. However, long term stability of NPs in the brain is still not well known and needs to be studied in more detail, especially in the case of cell-targeted nanoparticles. Further development has even led to magnetothermal control of silencing in neuronal circuits (Munshi et al., 2018; Liu et al., 2022). The wider adaption of magnetothermal neuromodulation, however, is still hindered by the need for sophisticated high power electronics to deliver the alternating magnetic fields with the necessary amplitude and frequency to the behaviorally relevant volumes, and by the limited temporal resolution because of the required heating (Munshi et al., 2017).

In this review, we will address current magnetic neuromodulation approaches and present advantages and limitations of using different magnetic stimulation approaches with focus on magnetothermal and magnetomechanical schemes. We will describe physical origins of heat dissipation and mechanical forces under external magnetic fields that are transduced from magnetic actuators to thermosensitive and mechanosensitive ion channels, main targets for magnetic activation or silencing of neuronal activity.

THERMOSENSITIVE AND MECHANOSENSITIVE ION CHANNELS

Thermosensitive ion channels

Temperature sensation is vital for the survival of living beings as biochemical reaction rates change with temperature. Specific temperature-sensitive ion channels belong to the large family of Transient Receptor Potential (TRP) channels first identified in nematodes. Most of these also exist in mammals where they underlie pain and temperature sensation. TRPV1 (Figures 3A and 3C), the first temperature specific channel cloned (Caterina et al., 1997), has an activation threshold of 41°C , and a temperature coefficient Q_{10} , a measure of how much a 10°C temperature increase changes the likelihood that a channel is open at a given time, of more than 20 (Cao et al., 2013). Several other members of the TRP superfamily have been found to have specific temperature thresholds, from cool to hot, with some of them being cold-sensitive, meaning that they open when the temperature drops below the threshold (Dhaka et al., 2006).

Recently, it has been discovered that some ion-channels respond to changes in temperature rather than a specific temperature threshold. One example is dTrpA1, a member of the TRP family found in flies (Hamada et al., 2008). Another are the TWIK-related potassium channels TREK1, TREK2, and TRAAK, which are activated by increases above physiological temperature (Schneider et al., 2014). These are also weakly mechanosensitive. In addition, some ion-channels with a different primary activation mechanism, have a

Table 1. Comparison of existing neuromodulation technologies

Technology	Delivery mechanism	Cellular Mechanism/ Ion Channel	Invasiveness of stimulus delivery	Temporal precision	Genetic modification	Celltype targeted	Large area or multiple regions	Multi-plex
Optogenetics (direct)	Implanted optical fiber or micro-LED	Photosensitive receptors and Ion-channels	High	msec	Required	Yes	Limited, requires multiple implanted fibers	yes
Optogenetics (IR)	Transcranial IR & Injected up-conversion NPs	Photosensitive receptors and Ion-channels	Minimal	msec	Required	Yes	Easy as NPs may be injected in multiple areas	No
Chemogenetics	CNO (Administered Intracranially, systemically or orally) binds to DREAAD	Engineered receptor (ligand gated channels)	None.	min - hours	Required.	Yes	Easy to create <i>trans-gene</i> in multiple areas	No
Transcranial Magnetic stimulation	Pulsed magnetic field causes electric field	Endogenous voltage sensitive channels	None.	>10 ms	No.	No	No. The focus is rather large.	No
Focused Ultrasound	Acoustic wave caused pressure and heat	Unclear mechanism	None.	<sec	No.	No	No. The focus is rather large.	No
Magneto-thermal	Injected NPs convert magnetic field energy into local heat	Thermosensitive channels	Minimal	<10 s	Not always required	Yes	Easy as field is globally applied, NPs delivered to multiple sites	Yes
Magneto-mechanical	Injected NPs convert magnetic field energy into force or torque	Mechanosensitive channels	Minimal	<sec	Not always required	Yes		?
Magnetoelectric	Injected NPs convert magnetic field energy into electrical field	Endogenous voltage sensitive channels	Minimal	>10 s	No	No		?

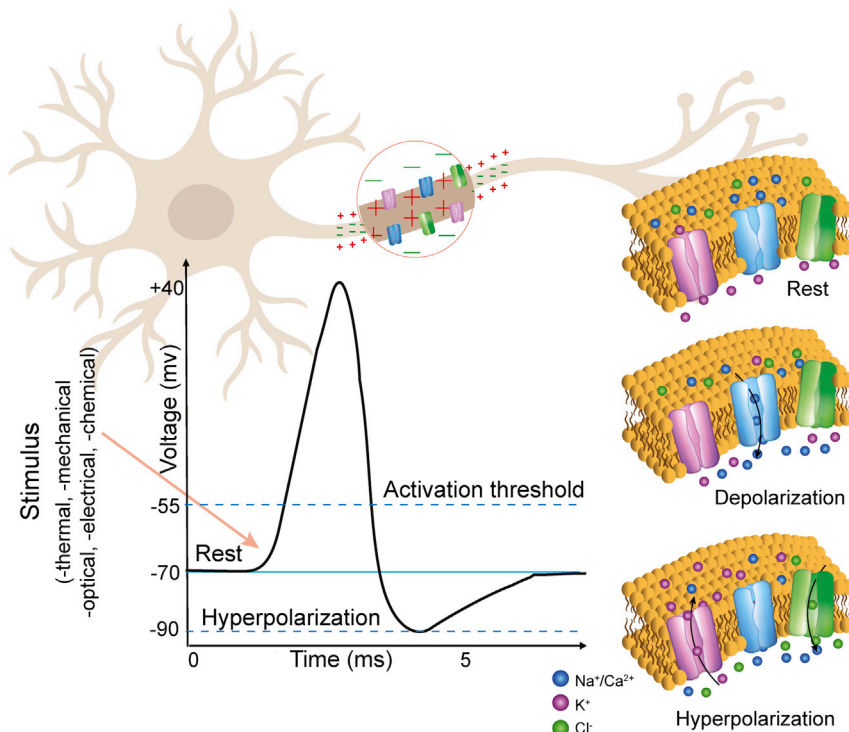


Figure 2. Principle of neuromodulation

Electrical activity in the brain is the result of an unbalanced distribution of ions inside and outside of the neurons. Electrical pulses, action potentials, are generated by ion-channel activity. Ion channels are transmembrane proteins with selective ion permeability that open pores in response to specific stimuli. For a resting neuron, the cell inside is 70 mV more negative than the outside. When the difference is reduced to 55mV or less, voltage gated Na^+ channels open, depolarizing the neuron until the voltage is positive enough to open K^+ channels which repolarize the neuron, ending the action potential. Most neuromodulation is based on additional ion-channels which when activated either shift the potential closer to the activation threshold or hyperpolarize the cells to suppress activity, such as Cl^- channel.

temperature sensitivity large enough to make them candidates for thermal neuromodulation, e.g., the Chloride channel TMEM16A (Munshi et al., 2018). The locations of the temperature-sensitive element of the various channels are still unknown and subject of several research efforts.

Mechanosensitive ion channels

Mechanosensitive ion channels (MSCs) convert mechanical stimulus to electrical or chemical activity. Native mechanical stimulation occurs in form of touch and pressure sensation (Santos-Sacchi and Dilger, 1988), changes in osmotic pressure, stress due to the blood flow, and bone structure. MSCs are essential for the development of tissues and organs (Soattin et al., 2016), as well as the differentiation of neuronal stem cells (Pathak et al., 2014). Cellular mechanosensation was discovered 40 years ago (Guharay and Sachs, 1984), but the first mechanospecific mammalian receptors, Piezo1 and Piezo2 (Figures 3B and 3D), were identified only a decade ago (Coste et al., 2010). The importance of this discovery was honored with the 2021 Nobel Prize. Other channels, such as the potassium channels TREK-1 and TRAAK, and a TRP member of vanilloid family 4, TRPV4, also respond to pressure changes (Strotmann et al., 2000; Liedtke et al., 2000; Grant et al., 2007). However, these channels are polymodal and often respond to other stimuli stronger. The Piezo1 channel responds to changes in lipid membrane tension and curvature, and is closed at low tension and high curvature. It appears that no interaction with the cytoskeleton of the cell is necessary to open the Piezo1 channel (Gottlieb et al., 2012). Other channels, such as TREK1 and TRAK likely respond to forces from multiple elements. Integrins sense in particular the tension from the cytoskeleton onto the substrate or neighboring cell. Piezo channels appear to be expressed in essentially all cells, including neurons across the central nervous system (CNS) at various expression levels. The exact details of expression level of all of these channels are subject of investigation.

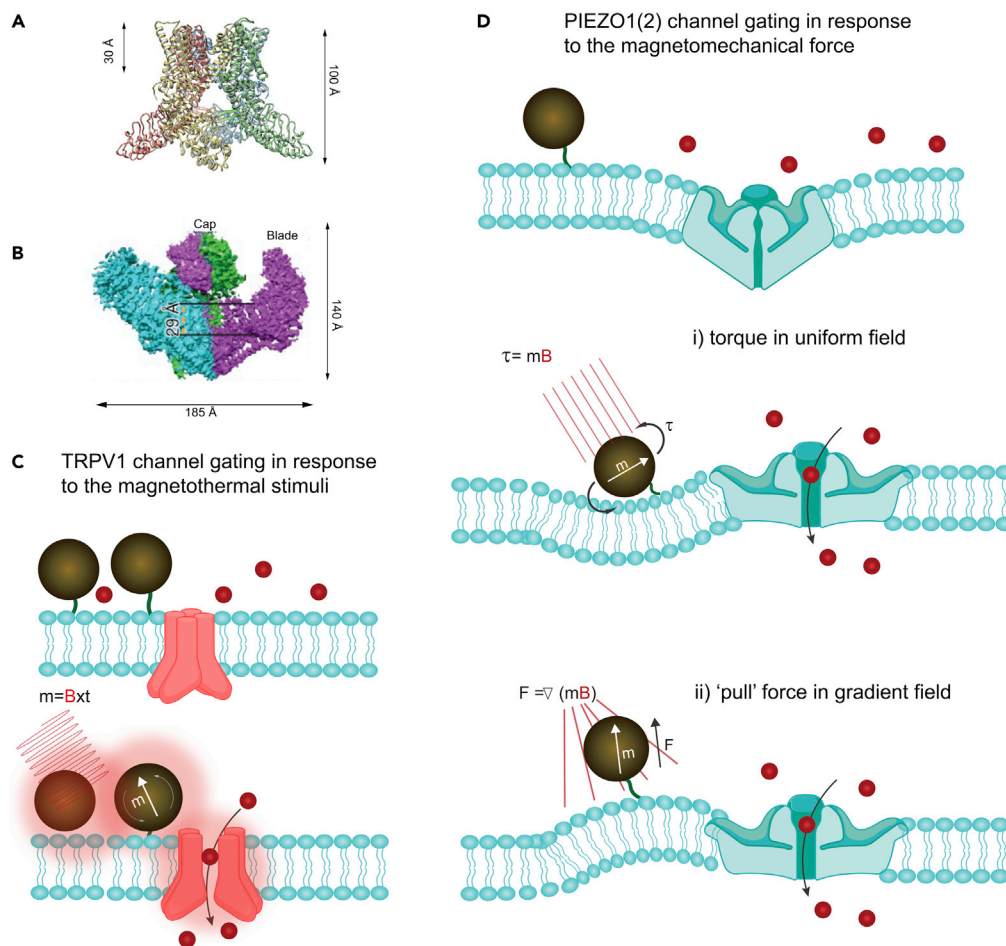


Figure 3. Magnetic activation of thermosensitive and mechanosensitive ion channels

(A) Electron cryomicroscopy determined structure of TRPV1 (Adopted from Liao et al.(Liao et al., 2013)), and (B) of mechanosensitive PIEZO1 channel (Adopted from Zhao et al.(Zhao et al., 2018)).

(C) Thermally gated ion channels, such as TRPV1 are activated when surrounding magnetic nanoparticles exhibit hysteretic heating under magnetic fields alternating at >100 kHz.

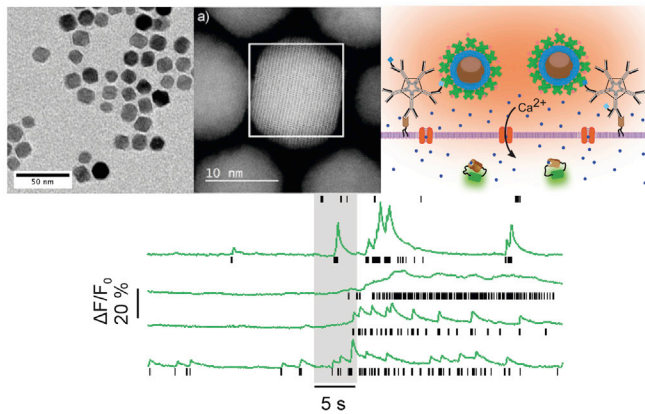
(D) Mechanosensitive channels, such as PIEZO1&2 can be activated with magnetic forces applied to the membrane portion in vicinity of the channel either by (1) torque under uniform fields or by (2) 'pulling' nanoparticle-tethered membrane under magnetic field gradients.

MAGNETOTHERMAL NEUROMODULATION

A decade ago, it was shown that remote magnetothermal activation of neurons is feasible by using magnetic nanoparticles to convert the magnetic field energy into heat to trigger the temperature-sensitive channel TRPV1 (Huang et al., 2010). This requires superparamagnetic nanotransducers, nanoparticles made of materials which would be ferro- or ferrimagnetic at larger scale, but are sufficiently small so that their magnetization thermally fluctuates. The nanoparticles are easily magnetized by an external magnetic field. When the direction of the external field is switched quicker than the particle's natural relaxation time, the repeated reversal of their magnetization causes hysteric losses which dissipate as heat (Figure 3C). By tuning the magnetic properties and size of the nanoparticles it is possible to optimize their response to specific frequencies of the applied magnetic field (Zhang et al., 2015), and to stimulate two populations with different frequencies (Moon et al., 2020).

So far, all efficient nanoheaters have been lab synthesized with the exception of particles isolated from magnetotactic bacteria (Hergt et al., 2005). The inorganic nanoparticles are encapsulated and coated with hydrophilic moieties to stabilize them in physiological solution. Then, they are either conjugated with peptides that target them to the membrane of neurons or they are pacified with polyethylene glycol

A Magneto-thermal stimulation with Co/Mn doped ferrite nanoparticles



B Magnetomechanical stimulation with magnetite nanodiscs

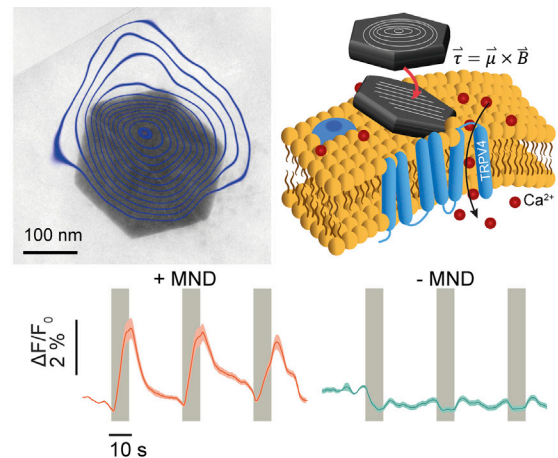


Figure 4. Principle of nanoparticle-based neuromodulation approaches

(A) Magneto-thermal stimulation with magnetic nanoparticles bound to membrane in vicinity of TRPV1 channel. Top: TEM micrographs of nanoparticles and schematic of Ca²⁺ influx in response to NeutrAvidin-Biotin membrane conjugated particles. Bottom: fluorescence traces from Ca²⁺ imaging (Adopted from (Munshi et al., 2017)).

(B) Magnetomechanical stimulation with magnetite nanodiscs. Top: Electron holography of vortex exhibiting MND. Ground vortex magnetization in MNDs aligns with the field direction causing exertion of torque that bends the membrane and opens the mechanosensitive ion channel. Bottom: At 5 Hz, 30 mT MNDs immediately and repeatedly cause Ca²⁺ influx in mechanosensory DRG neurons (Adopted from (Gregurec et al., 2020)).

(PEG) to act as suspension. As the heating spreads in a small volume, the magneto-thermal transducers need to be close to the thermosensitive channels but do not have to be directly coupled to them. Targeting the nanoparticles to the cell membrane leads to localized heating with rapid cooling to shut off the signal after the magnetic field is turned off. Using a suspension of nanoparticles causes a larger volume to heat more robustly at the cost of slower cooling (Figure 4A). A suspension of nanoparticles injected in the brain can remain localized and be functional for many months (Chen et al., 2015). In the rodent brain the heating is localized to a volume less than 10 aL, or 0.01 μm³ (Munshi et al., 2017; Liu et al., 2022). Heating and cooling times of 1 s in cell culture and 10 s in freely moving rodents have been achieved (Munshi et al., 2017). Although improvement in amplifier and coil design may reduce the lag time further, the temporal control is limited by the fact that activation of TRPV1 requires tissue heating of 2–3°C. Using channels which respond to sudden temperature changes rather than a threshold temperature offer potentially a much-increased response time. One demonstration of this is the magneto-thermal stimulation of dTrpA1 to evoke a sub-second behavioral response in *Drosophila* (Figure 5D) (Sebesta et al., 2021). Using TREK1 potassium channel provides magneto-thermal neuronal silencing with similarly decreased response time (Figure 5C) (Liu et al., 2022).

Magneto-thermal neuromodulation has been performed using cell membrane targeted nanoparticles or a small injected bolus of nanoheaters because isolated nanoparticles lose their heat to the environment too quickly. *In vitro* studies showed that the heating produced by cell membrane attached nanoparticles is substantial at the membrane, scales with the area density of nanoparticles, but undetectable by a nearby thermal probe in the media (Munshi et al., 2017). In contrast heating of a suspension of nanoparticles scales with the volume density of nanoparticles and is homogeneous (Munshi et al., 2017; Davis et al., 2020). The thermal spread in the living brain is less studied and understood. Recent experiments demonstrated that the heating of a bolus of nanoparticles in the brain may be confined to a small volume. Liu et al. injected 1 μL of 10mg/mL nanoheaters and measured that the temperature change in the nearby neurons dropped quickly with distance, so that cells further than 0.3 mm wouldn't be affected (Liu et al., 2022).

MAGNETOMECHANICAL NEUROMODULATION

Magnetic nanomaterials can transform the energy of the magnetic field into mechanical energy to actuate mechanosensitive cells (Figures 3B and 3D). Mechanosensitive ion channels are gated by forces in the order of tens to hundreds of piconewtons (Howard and Hudspeth, 1988; Moe and Blount, 2005; Wu et al.,

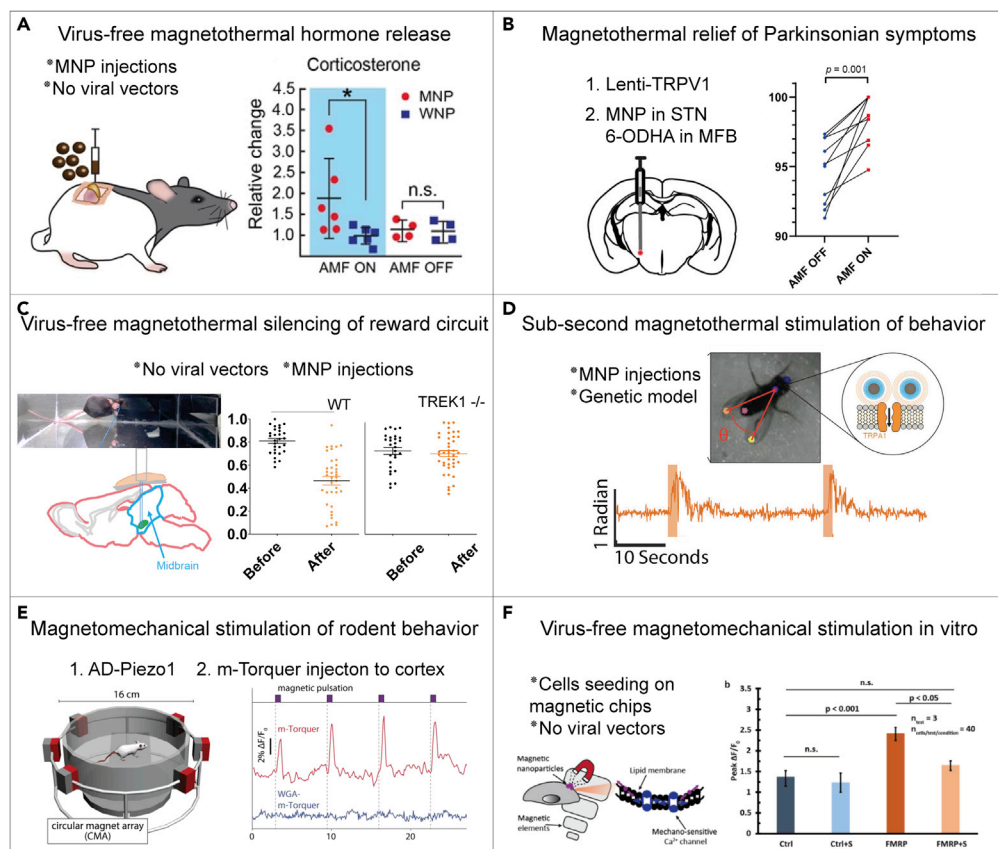


Figure 5. Application examples of magnetic nanoparticle-based neuromodulation

(A) Virus-free, magnetothermal stimulation of mouse adrenal gland via endogenous TRPV1. Left: Surgical procedure for injection of MNPs into the rat adrenal gland. Given the endogenous expression of TRPV1 in the adrenal medulla and cortex, there is no need for viral vectors. Right: Normalized relative change in corticosterone concentrations following AMF stimulation as compared to the baseline level for each rat before AMF (adopted from (Rosenfeld et al., 2020)).

(B) Magnetothermal stimulation of dopamine release to relief Parkinson symptoms. Left: Surgical procedure for direct injection of MNPs into the subthalamic nucleus of 6-hydroxydopamine (6-OHDA) hemiparkinsonian mice. Subthalamic nucleus neurons were heat-sensitized by lentiviral delivery of TRPV1 7 weeks earlier. Right: representative CatWalkXT results. AMF stimulation resulted in increased step sequence regularity (%) (adopted from (Hescham et al., 2021)).

(C) Virus-free, magnetothermal silencing of mouse VTA via endogenous TREK1. Left: Mouse in the light/dark box, in which the dark compartment is covered by the AMF coil. Magnetothermal silencing using endogenous TREK-1 channels can suppress dopaminergic activity in the ventral tegmental area. Fiber photometry was used to determine the temperature at the MNPs and target neurons. Right: Ratio of time spent in the dark compartment for wild-type (WT) and TREK1 knock-out mice before and after AMF stimulation (adopted from (Liu et al., 2022)).

(D) Sub-second magnetothermal stimulation of fly behavior using TRPA1. Top: Wing opening due to magnetothermal activation of TRPA1. Bottom: Wing angle plots as a response to thermal stimulation and schematic of behavioral chamber (adopted from (Sebesta et al., 2021)).

(E) Magnetomechanical stimulation of rodent behavior. Left: Schematic of PIEZO1 opening via rotational force. Right: Ca²⁺ influx in stimulated neurons and schematic of stimulation arena (adopted from (Lee et al., 2021)).

(F) Virus-free magnetomechanical stimulation *in vitro*. Left: Two-week-old cortical neurons seeded beside magnetic elements with high local field gradients are stimulated with MNPs and a permanent magnet to induce calcium influx. Right: Magnetic forces were able to restore physiological expression levels of inhibitory GABA_A receptors in a fragile X syndrome neural network model following chronic stimulation. “S” refers to chronically stimulated and “FMRP” to fragile X mental retardation protein (adopted from (Tay and Di Carlo, 2017)).

2016; Liu et al., 2022). The forces produced by the magnetic transducer should be sufficiently large to activate mechanoreceptors but below thresholds that may disrupt the anatomy and physiology of cellular membrane. In available techniques, the transferred forces range from femtonewtons to piconewtons (Seo et al., 2016; Tseng et al., 2012; Lee et al., 2014, 2021; Gregurec et al., 2020; Tay et al., 2016). For efficient force transduction the actuator should be directly coupled to the mechanosensitive ion channel. This could

be achieved by binding the nanomaterials via antibodies or genetically engineered tags on the channel (see Biofunctionalization section for further details) (Yu et al., 2022; Hughes et al., 2008). However, the location of where the nanoparticle should be attached is often not known, as the details of force sensation of many mechanosensitive ion-channels are not yet well understood.

Early attempts of mechanical manipulation used uniform magnetic fields which rotate ferromagnetic nanoparticles acting as nanomagnets to align with the field direction. This causes a torque, ($\vec{\tau} = \vec{\mu} \times \vec{B}$, where $\vec{\mu}$ is the dipole moment and \vec{B} is the magnetic field), on the membrane or target molecule. However, static uniform fields cannot produce any translational force on a single nanomagnet. Yet, superparamagnetic particles with <100 nm inter-distance may experience a magnetic dipole-dipole attractive force in uniform fields which leads to particle aggregation on a cell membrane.

A magnetic field gradient is necessary to exert lateral forces on isolated nanomagnets. Magnetic tweezers (Gosse and Croquette, 2002) using micrometer paramagnetic beads in a gradient field that generate nanonewton forces have been a useful tool for single molecule biophysics. However, the large size of the transducer limits this approach when applied to cells where proteins are packed densely (Amblard et al., 1996). Newer concepts using clustered (Mannix et al., 2008) or protein targeted (Seo et al., 2016) magnetic nanoparticles produce weaker forces, fN-pN, but allow single channel mechanical manipulation. (Kim et al., 2018) Wu et al. used such a magnetic tweezer design to pull on streptavidin coated nanoparticles linked to biotinylated Piezo1 channels in cells (Wu et al., 2016). That work demonstrated that applying a force of ~10 pN to the putative mechanically sensitive domain of Piezo1 perturbs the mechanical gating.

However, it is not necessary to couple nanomaterial directly to the mechanosensitive channel to stimulate neurons. Tay et al. showed that neurons grown on hydrogel in which nanoparticles were embedded, are robustly stimulated by applying a magnetic field gradient (Figure 5F) (Tay and Di Carlo, 2017).

More recent magnetomechanical approaches use sophisticated anisotropic nanomaterial designs that allow for long distance control of force transmission with slowly alternating uniform magnetic fields (<20 Hz) (Figures 4B and 5E). Particles of sizes between single-domain and multi-domain, can exhibit a magnetic vortex ground state, in which magnetic flux closure is achieved without domain walls. The magnetic vortex configuration has a characteristic circular arrangement of spins, especially likely in disc-shaped particles made of magnetic materials with high saturation magnetization which drives the minimization of the magnetostatic energy. Low magnetocrystalline anisotropy and low exchange stiffness constant allow the circular configuration of spins without large energy penalties (Parreiras and Martins, 2015). Due to the minimization of energy, the vortex nanoparticles in ground state exhibit near-zero net magnetic moment reducing the dipole-dipole interactions of the particles thus strongly improving their colloidal stability.

When external magnetic fields (MFs) are applied, anisotropic nanoparticles assume in-plane spin alignment, and the resulting magnetic moment can be used to apply mechanical torques to the cell membranes. This inspired biomedical applications of Permalloy microdiscs for apoptosis of cancer cells relying on torsional force destructive to the cellular membranes (Kim et al., 2010). However, Permalloy microdiscs are not suitable for neuromodulation due to their large size and low stability from oxidation under physiological conditions. Recently, Gregurec et al. demonstrated magnetomechanical stimulation using biochemically stable, vortex exhibiting anisotropic magnetite nanodiscs (MNDs, ~100–250 nm diameter) as transducers of torques to mechanosensory cells. When coupled to a weak, slowly varying magnetic field, MNDs transitioned between vortex and in-plane magnetization delivering torques sufficient to trigger mechanoreceptors without disrupting membrane integrity (Figure 4B). Using magnetic fields of <30 mT and <10 Hz allowed for pulsed control of Ca^{2+} influx in dorsal root ganglia sensory neurons. The intensity of the response decreases with the MND size. The mechanical stimulus delivered to the cell membrane is tunable via the magnetic field frequency and amplitude (1–10 Hz, 10–30 mT). MNDs also activated TRPV4 ion channels expressed in otherwise non-mechanosensitive HEK-293 cells. The simplicity of the magnetic field apparatus, high-throughput hydrothermal synthesis of hematite nanodiscs templates with subsequent reduction to vortex confining magnetite phase, and tunability of mechanical stimuli makes this approach a promising nanotechnology-based platform for emerging bioelectronic medicines (Gregurec et al., 2020). Recently, Lee et al. created a nanoactuator, the ‘m-Torquer’, by assembling octahedral magnetic nanoparticles on the surface of a 500 nm non-magnetic bead. The m-Torquer follows the rotation of a weak, rotating

uniform field to deliver torques from 2–50 pN. When tethered to Piezo1, these torques were sufficient to trigger the channel opening (Figure 5E). The group applied this approach in the motor cortex of mice and demonstrated activation at distances of up to 70 cm (Lee et al., 2021). The demonstration of long-range and precisely tuned mechanical stimulus on mechanosensitive ion channels by these groups, started new studies to explore macroscale anisotropic materials for non-invasive mechanotransduction (Collier et al., 2022), and are used as robust tools to explore endogenous mechanosensitivity in the brain (Su et al., 2021).

BIOFUNCTIONALIZATION OF INORGANIC NANOPARTICLES

Nowadays there are different established approaches to prepare magnetic nanomaterials and composites with controlled physical and chemical properties. For more details on the synthesis, we refer the reader to several recent reviews (Anik et al., 2021; Liu et al., 2020; Zamani Kouhpanji and Stadler, 2020).

Main prerequisite for any biological application of inorganic nanoparticles is the proper surface chemistry and functionalization. Surface chemistry will determine interactions that occur in the biological environment, control the cellular uptake, shape the colloidal stability and allow for the targeting of the nanoparticles to the biological entity via appropriate functional groups and molecules on the surface. Precisely tailored surface chemistry will enable particles to avoid formation of protein corona-adsorption of immune proteins on the surface of nanomaterial once introduced in the biological environment, presenting a physical and biochemical barrier between the nanoparticle and targeted entity. Protein corona may lead to immune responses, acquires the particles to aggregation and hinders the transfer of the physical stimulus to the targeted moiety (Caracciolo et al., 2017). Tailored post synthesis surface chemistry allows for biocompatible and colloidally stable nanoparticles and prevents formation of protein corona on the nanoparticle surface. Lipids (Jiang et al., 2013) or amphiphilic polymers (Pellegrino et al., 2004) such as polyethylene glycol (PEG) successfully stabilize magnetic nanoparticles and reduce ability of cellular uptake. PEG used in combination with polymaleic acid anhydride alt-1-octadecene (PMAO) allows for coating of ligand stabilized particles via hydrophobic interactions and their transfer to the water phase (Moon et al., 2020). PEG polymers also serve as a versatile platform for bioconjugation and chemical modification for binding of biological molecules to the polymer coating.

Targeting of nanoparticles to specific cells or to specific molecules requires further bioconjugation. The Biotin-streptavidin complex is one of the oldest popular tagging systems for conjugation of biomolecules. Huang et al. leveraged biotinylated AP membrane domain to link streptavidin coated magnetic nanoparticles which in turn allowed for targeted heating of TRPV1 (Huang et al., 2010). His-tag, SNAP-tag and HALO-tag chemistries allow for similar conjugation. The His-tag requires the smallest insertion into the target protein sequence, just 6 histidine residues. The magnetic particles coated with anti-His antibody can be targeted against the His-binding motifs. His tag inserted in modified TREK1 channel allowed for particle mediated mechanical manipulation (Hughes et al., 2008). The SNAP- and HALO-tag require larger insertions of enzymes which then permit highly specific labeling using a very small moiety. In addition, antibodies against endogenous proteins have also been used to route nanoparticles to the membrane proteins. Carvalho-de-Souza et al. conjugated Ts1 neurotoxin to the surface of Au nanoparticles which allowed for specific targeting of voltage gated ion channels and thermal modulation of membrane capacitance (Carvalho-de-Souza et al., 2015). Variety of the targeting chemistries available together with selection of magnetic nanoparticles capable of producing thermal or mechanical stimulus may now be used in combination as a powerful tool in neurobiological studies.

APPLICATIONS OF MAGNETIC NEUROMODULATION

Neuromodulatory interventions as therapeutic approaches to neurological and psychiatric disorders require upregulation and/or downregulation of activity of specific neural circuits. Historically, only downregulation via surgical lesions could be accomplished. In the late 20th century, the neurosurgical treatment toolbox for refractory neurologic and psychiatric disorders was increased by deep brain stimulation (DBS) (Temel and Jahanshahi, 2015). DBS is based on the stereotactic implantation of quadripolar electrodes delivering current in specific subcortical structures. It is usually indicated to decrease symptoms in neurological and psychiatric disorders, such as Parkinson's disease, essential tremor, dystonia, obsessive-compulsive disorder and other neuropsychiatric disorders (Deuschl et al., 2006; Krauss et al., 1999; Benabid et al., 1991; Hubble et al., 1996). A main drawback of current DBS is the required chronically implanted electrode lead connecting to the stimulator outside of the brain, which makes many patients reluctant to undergo DBS even when treatment is warranted (Lange et al., 2017). Recent advances in the field of neuromodulation stress the urgency of developing wireless deep brain stimulation treatments (Temel and

Jahanshahi, 2015). An alternative approach is the use of magnetic nanomaterials, which activate temperature- and/or mechanosensitive Transient Receptor Potential (TRP) channels such as TRPV1 and TRPV4, and Piezos to enable control of cell activity with a magnetic field (Huang et al., 2010; Chen et al., 2015).

Finally, a first study with translational implications investigated whether magnetothermal stimulation of the subthalamic nucleus (STN) can provide therapeutic benefit in a mild and severe parkinsonian mouse model. The authors found that motor symptoms of parkinsonian mice injected with NPs became comparable to controls when exposed to an AMF (Figure 5B) (Hescham et al., 2021).

Magnetomechanical neural stimulation has been demonstrated *in vitro* via patch clamp studies (Hughes et al., 2008) and calcium imaging (Lee et al., 2014). Researchers have also demonstrated the utility of chronic magnetomechanical stimulation to restore mechanosensitive ion channel equilibrium in fragile X syndrome model neural networks (Figure 5F) (Tay and Di Carlo, 2017). The aforementioned studies, however, have relied on devices similar to magnetic tweezers (Seo et al., 2016) that generate high magnetic field gradients on the order of 100 mT. Therefore, cells need to be seeded in close proximity to the magnetic elements with high local field gradients to achieve neuromodulation with magnetic nanoparticles. For potential *in-vivo* applications and to allow deep tissue penetration, a high magnetic gradient implant has to be created such as an endovascular stent-magnetic array (Oxley et al., 2016). Contrary to this, a recent study demonstrated the applicability of magnetic nanodiscs, which act as transducers of torque, for remote control of mechanosensory neurons in weak (≤ 28 mT), low frequency magnetic fields (Gregurec et al., 2020). In particular, magnetomechanical activation of the mechanosensitive cation channel TRPV4 exogenously expressed in HEK293 cells and stimulation of hippocampal neurons resulted in an increase of intracellular Ca^{2+} concentrations. With regard to peripheral stimulation, magnetomechanical control has been demonstrated in dorsal root ganglions (DRGs), which endogenously express a wide range of mechanoreceptors, such as Piezo2 and TRPV4 (Gregurec et al., 2020). Future studies could investigate whether magnetomechanical stimulation of DRGs can be employed for the treatment of chronic pain and pose a wireless alternative to electrical DRG stimulation (Huygen et al., 2020). Magnetic coils suitable for *in vivo* magnetomechanical neuromodulation using these magnetic nanodiscs can be engineered more readily to efficiently generate appropriate magnetic field conditions. The majority of studies discussed above require the expression of virally-transduced TRPV1 and TRPV4 to enable remote neuromodulation. This poses unclear safety consequences, especially regarding insertional mutagenesis and oncogene activation, and therefore represents a major hurdle to the translation into the clinic. That being said, however, a number of gene therapies have now been approved in Europe (Touchot and Flume, 2017) and the United States (Young et al., 2022) and many clinical trials are underway to test the safety and efficacy of gene therapies. Most of these approaches use similar viral vectors such as adeno-associated virus. TRPV1 and TRPV4 are also endogenously expressed in neurons and glia cells in the mammalian CNS and may cease the need to rely on transgenes in specific neuronal circuits. Further studies are warranted to investigate, whether the endogenous expression levels in clinically relevant brain targets are sufficient to enable magnetic neuronal control (Terzian et al., 2014; Starowicz et al., 2008; Marinelli et al., 2005; Sun et al., 2013; Nam et al., 2015).

CONCLUSIONS AND OUTLOOK

Over the past decade, magnetothermal neuromodulation has been developed from proof of principle in cell culture to a true wireless deep brain neuromodulation tool that is being used to discover neuronal pathways (Liu et al., 2022) and to develop novel translational therapies. The technique is sufficiently developed that it can be adapted by labs around the world. Magnetothermal neuromodulation provides the advantage of true wireless stimulation or silencing, the ability to multi-plex different signals, the capacity to simultaneously modulate large regions or multiple regions of the brain, to provide minute long silencing without side effects, and to be fully reversible. Earlier latency of 5–10 s have been reduced to sub-second with new temperature-sensitive channels and improved nanoparticles. These advantages are countered by a few limitations: Activation requires the genetic modification of the target neurons, but silencing can be done with endogenous channels. The nanoparticles need to be injected near the target. The power to generate alternating magnetic fields of the necessary frequency and strength limit the size of the coils and hence application area. Nanoparticle synthesis and power electronics have been largely optimized and standardized, and are commercially available.

Future research in magnetothermal neuromodulation will focus on discovery and design of ion channels which respond to a sudden change in temperature rather an absolute temperature threshold to reduce

the stimulation time to the speed of neurological signaling. Furthermore, there will be an effort to identify endogenous channels suitable for magnetothermal neuromodulation to enable gene-free magnetothermal neuromodulation, and with that a path to translational applications. The recent work by Liu et al. (2022) demonstrated both ideas by fast magnetothermal neuronal silencing via the endogenous channel TREK1 without appreciable temperature increase.

Magnetomechanical stimulation, although less developed than magnetothermal, has the potential to be adopted very broadly because generating the required magnetic fields is significantly easier. In the simplest case, cheap permanent magnets may be used. This also increases the area in which the field can be applied from around 10 cm available in magnetothermal approach to 1 m range in magnetomechanical, opening the methods for large animals and even humans. Future research in magnetomechanical stimulation will focus on the discovery and design of further mechanosensitive ion-channels and biological transducers, both genetically delivered as well as endogenous for gene-free applications. Also the design of specific tethers and targeting tools to optimally transmit the generated force on the biological mechanosensitive element will likely be a focus of interest.

Outside of the brain, magnetomechanical stimulation allows for robust and spatially detailed studies of the mechanotransduction in cells and animals. It also opens the avenues to build on the existing methods for treatment of pain or disorders of peripheral nervous system by targeting endogenous mechanosensitive channels.

Magnetothermal and-mechanical techniques provide wireless, cell specific control of CNS. Relying on delivery of thermal or mechanical stimulus to the genetically targeted CNS neurons diminishes the limitations of off-target effects of other wireless magnetic, ultrasound or electric stimulation. Some recent studies reveal some CNS circuits to endogenously express sufficient numbers of temperature or mechanosensitive channels that genetic manipulation is not necessary (Liu et al., 2022; Su et al., 2021). This opens up multiple translational possibilities. A final challenge for translational application may be the delivery of the magnetic nanomaterials into the brain. The experience with animals has demonstrated that the stereotactic injection is minimally invasive, but does bear the risks of hemorrhage or infection. A promising alternative delivery may be the focused ultrasound mediated transient opening of the blood-brain-barrier to deliver the nanoparticles to the target region (Hynynen et al., 2005).

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AUTHOR CONTRIBUTIONS

Conceptualization- D.G., S.H., A.P.; writing-original draft-L.S. and S.H.; writing-review & editing-D.G. and A.P.; supervision-D.G. and A.P.; Project administration- D.G., Lead contact- D.G.

DECLARATION OF INTERESTS

D.G. is among the inventors listed on US Patent 20200001104 "Scalable magnetomechanical schemes and devices for remote control of mechanosensitive cells".

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